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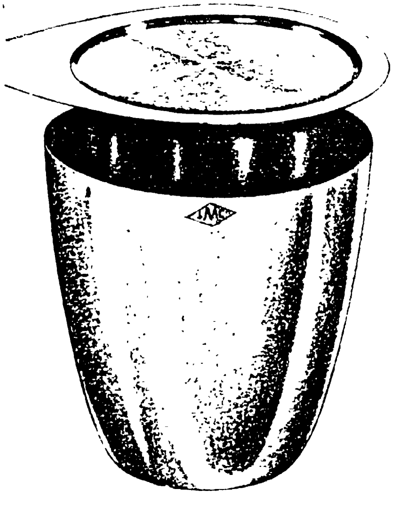

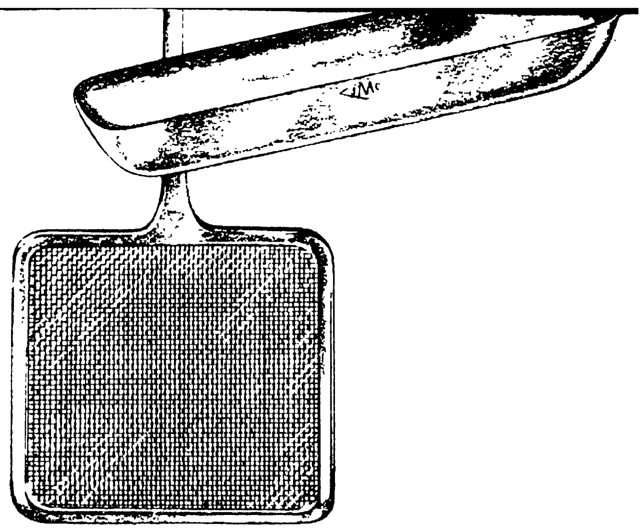

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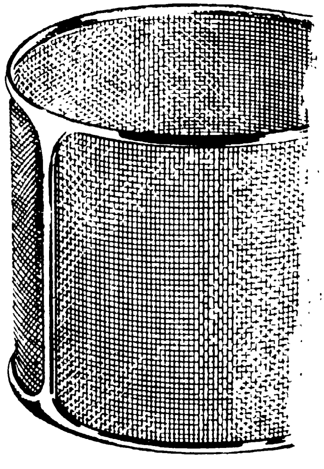

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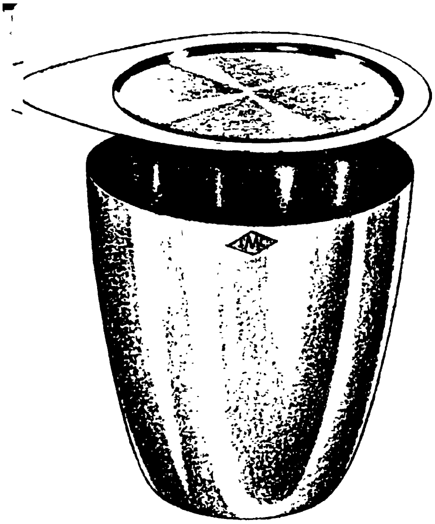

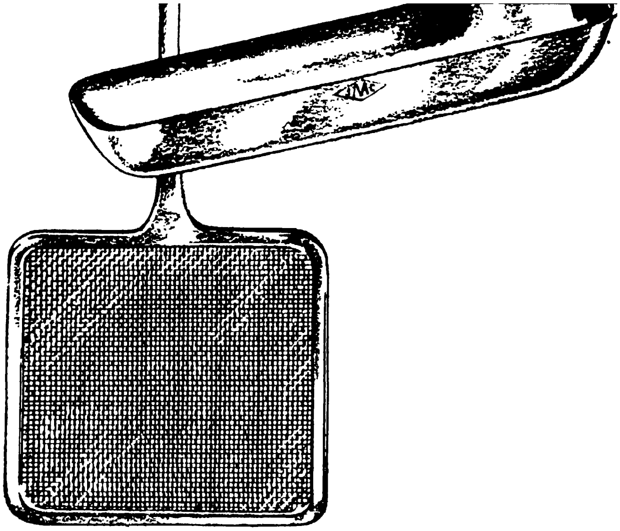

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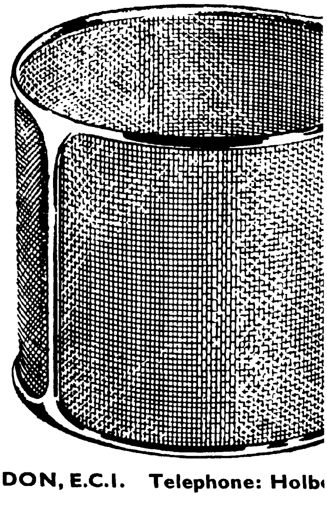

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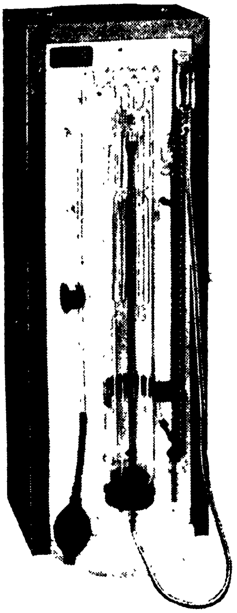

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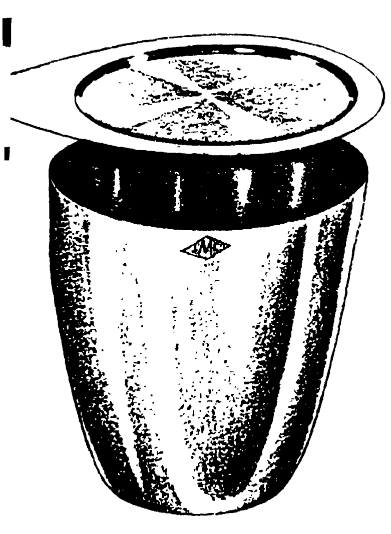

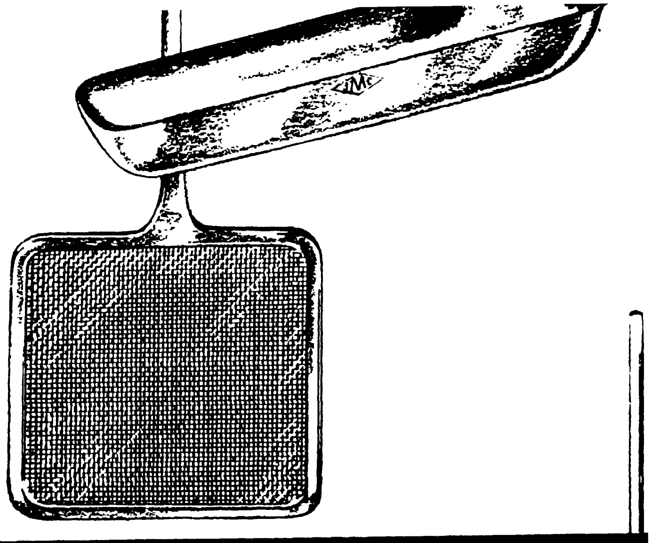

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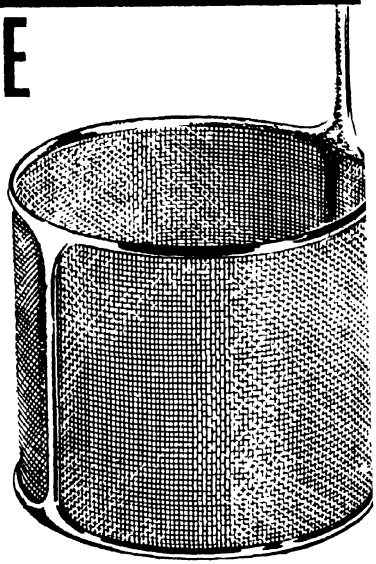

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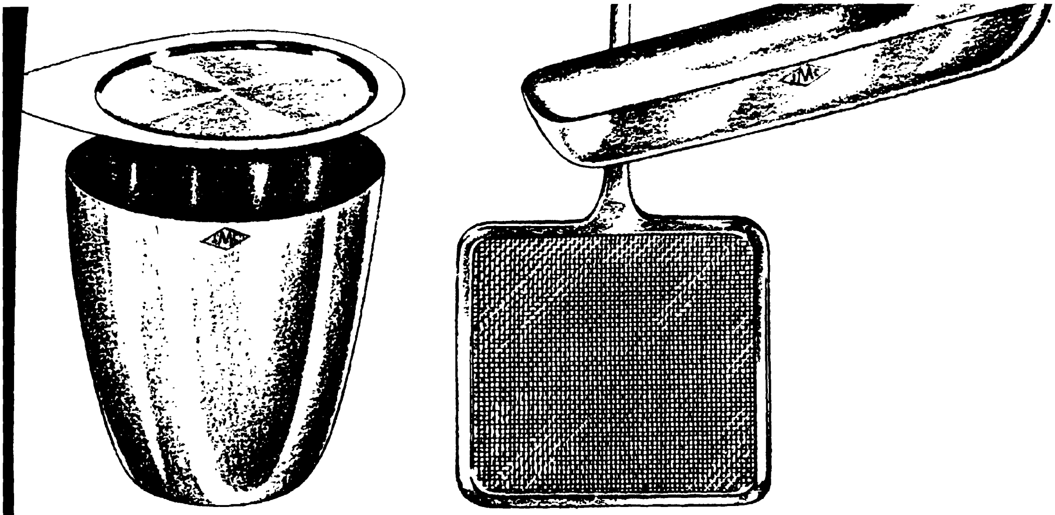

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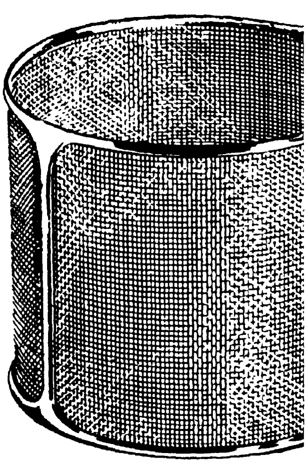

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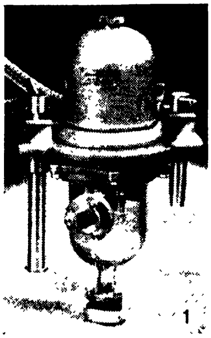

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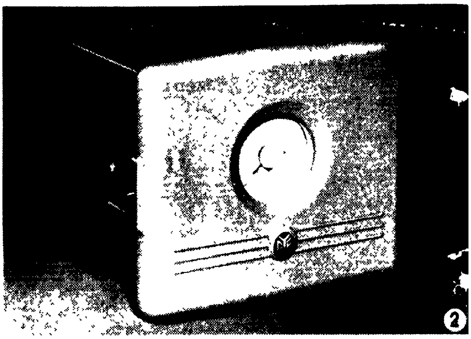

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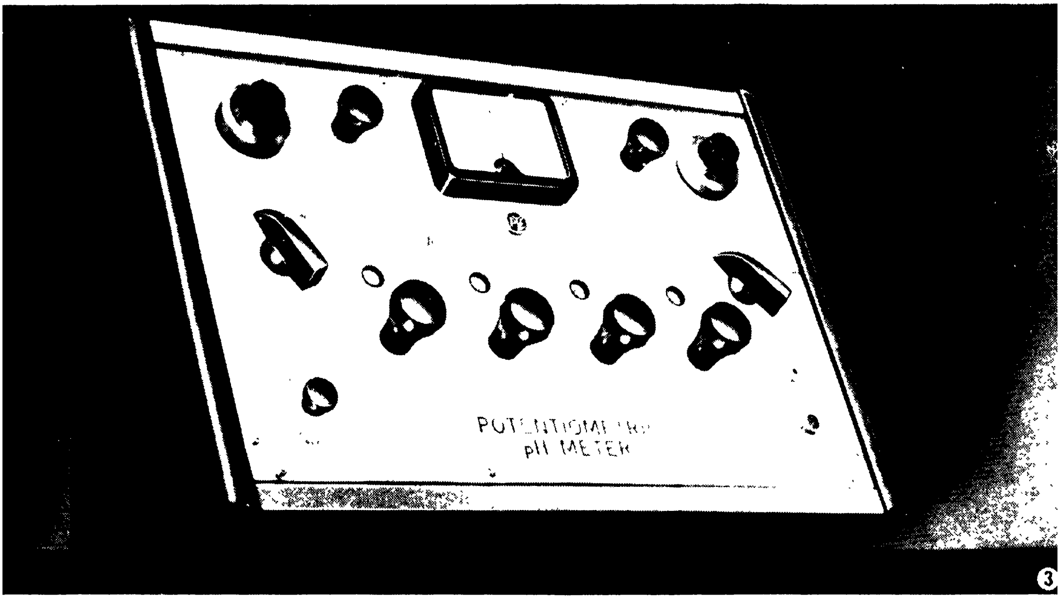

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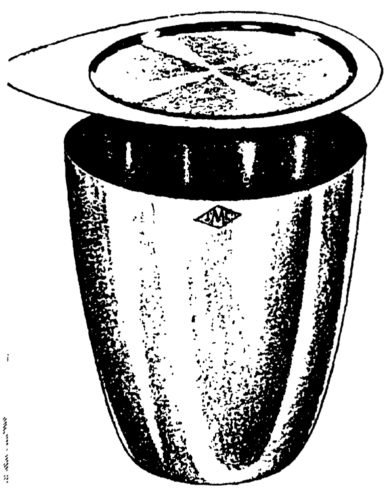

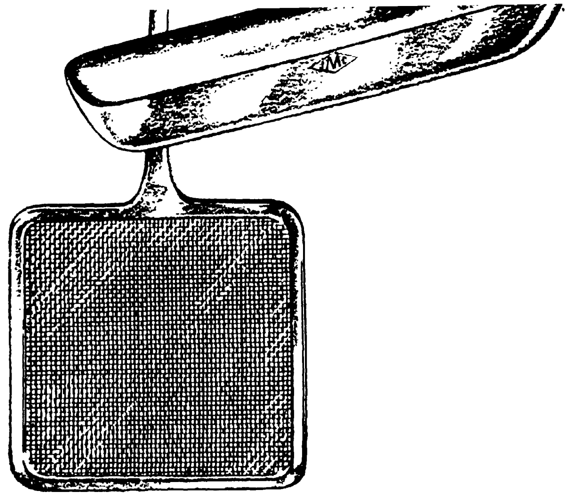

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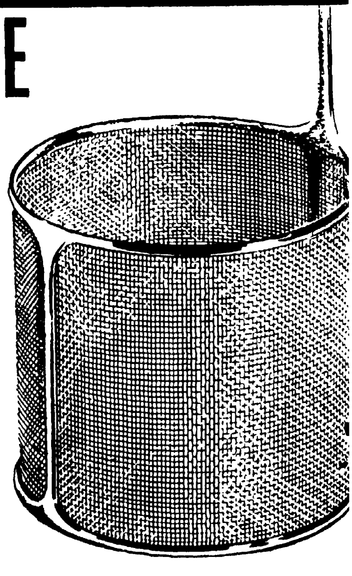

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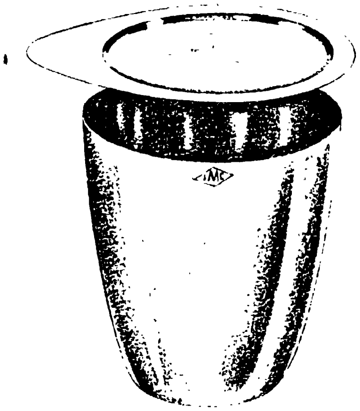

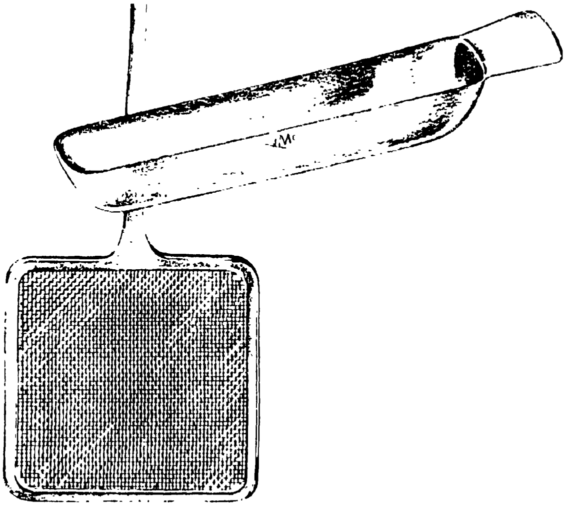

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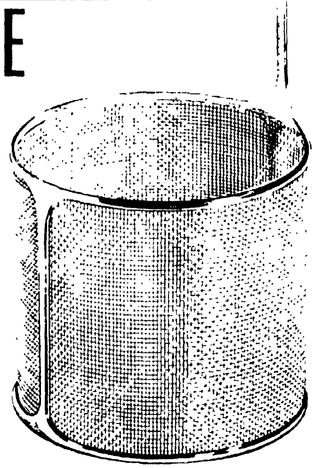

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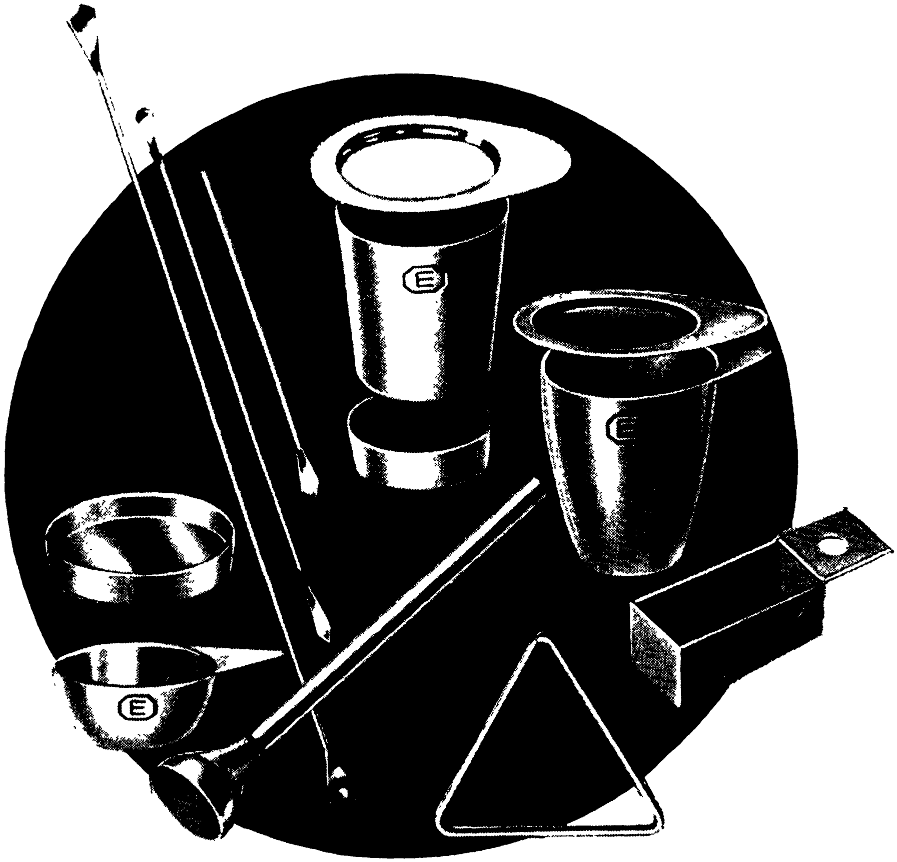

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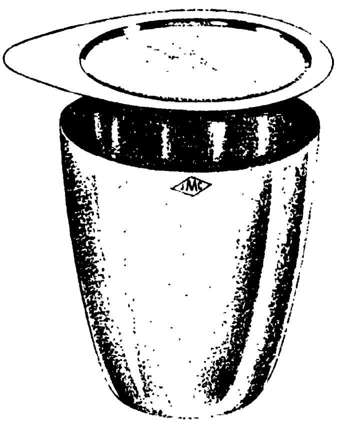

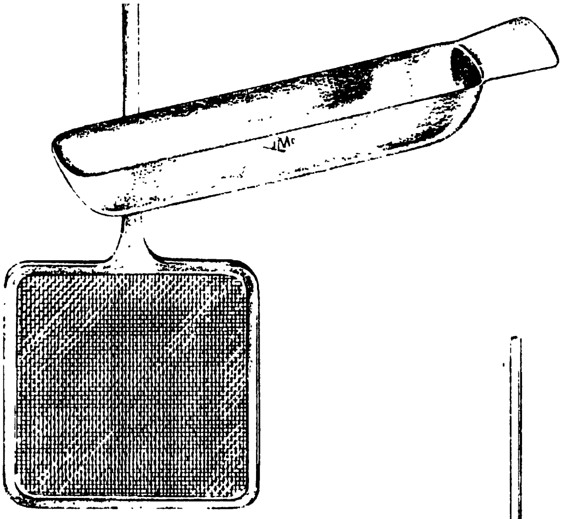

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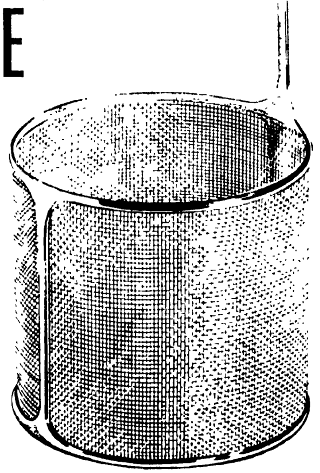

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JANUARY, 1963 Vol 88, No. 1042

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PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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ORDINARY MEMBERS

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JUNIOR MEMBERS

Arnold John Bloom, M.Sc.Tech. (Manc.); Helen Shirran Gilmour, B.Sc. (Aber.); John William Saich.

DEATHS

WE record with regret the deaths of

Robert Emrys Jones
William Joseph Sommerville Pringle.

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Physical Methods Group was held at 2.15 p.m. on Saturday, December 8th, 1962, at the Old Nag's Head Hotel, Lloyd Street, Manchester. The Chair was taken by the Chairman of the North of England Section, Mr. J. Markland, B.Sc., F.R.I.C.

A lecture on "Nuclear Magnetic Resonance" was given by Professor E. R. Andrew, M.A., Ph.D.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Chemical Society, the Society of Chemical Industry and the Royal Institute of Chemistry was held at 6 p.m. on Friday, December 7th, 1962, in Room 24, Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Glasgow Section of S.C.I., Professor J. Monteath Robertson, C.B.E., M.A., Ph.D., D.Sc., F.R.S.

The following paper was presented and discussed: "Thoughts on Meat," by E. C. Bate-Smith, M.Sc., Ph.D., M.Inst.R.

WESTERN SECTION

A JOINT Meeting of the Western Section with the Cardiff and District Section of the Royal Institute of Chemistry was held at 7 p.m. on Friday, December 7th, 1962, at University College, Cardiff. The Chair was taken by the Vice-Chairman of the Western Section, Mr. E. A. Hontoir, B.Sc., A.I.M.

The following paper was presented and discussed: "Gem Stones and Jewels, Natural and Synthetic," by R. C. Chirnside, F.R.I.C.

PHYSICAL METHODS GROUP

THE Eighteenth Annual General Meeting of the Group was held at 6.30 p.m. on Tuesday, November 27th, 1962, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Vice-Chairman of the Group, Mr. L. Brealey, B.Sc., F.R.I.C. The following appointments were made for the ensuing year: *Chairman*—Dr. W. Cule Davies. *Vice-Chairman*—Mr. L. Brealey. *Hon. Secretary and Treasurer*—Dr. T. L. Parkinson, Product Research Department, Beecham Food and Drink Division Ltd., Harpenden Rise, Harpenden, Herts. *Members of Committee*—Mr. B. B. Bach, Dr. J. A. W. Dalziel, Mr. J. H. Glover, Mr. W. C. Hanson, Mr. D. Moore and Mr. D. C. M. Squirrell. Dr. D. C. Garratt and Mr. C. A. Bassett were re-appointed as Honorary Auditors.

The Annual General Meeting was followed by the Eighty-fifth Ordinary Meeting of the Group, at which the subject of the meeting was "Recent Improvements in Separation Techniques" and the following papers were presented and discussed: "High-voltage Paper Electrophoresis," by D. Gross, Ph.D. (see summary below); "Solvent Extraction of Inorganic Compounds, with Special Reference to Synergism," by Professor H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., L.R.A.M.

HIGH-VOLTAGE PAPER ELECTROPHORESIS

DR. D. GROSS said that paper electrophoresis had proved very useful in the field of high-molecular compounds, particularly proteins. In the field of low-molecular compounds progress had been much slower. In order to be competitive with paper chromatography, paper electrophoresis had to offer definite advantages. This it did in an impressive way for proteins, but not markedly for low-molecular compounds, apart from the different sequence of positions.

The introduction of the high-voltage technique employing 5 to 10 kV or potential gradients of 100 to 200 volts per cm had changed the situation significantly. Reduction in running time and hence minimisation of adverse diffusion effects had turned paper electrophoresis into a rapid and convenient tool of a high resolving power, which by itself or as a complement of paper chromatography was proving extremely valuable in many analytical problems.

The lecturer then gave briefly the theoretical background to the development of the high-voltage technique and stressed the difficulties encountered in designing suitable equipment. The main points to be observed were efficient heat dissipation and overall pressure control, where required. He first dealt with various designs currently used and then described in more detail the equipment he had developed over the last 10 years, which resulted in an apparatus of the double-cooled sandwich type with highly reproducible pressure control. In several years of service it had proved to be reliable, convenient, robust and safe, and required surprisingly little maintenance.

The lecturer next discussed some results of the application of high-voltage paper electrophoresis to problems in the fields of inorganic and organic chemistry. Limitation of time did not allow him to refer to more than one or two examples for each group of compounds such as inorganic cations—particularly the alkali and alkaline-earth metals, complex inorganic anions—particularly oxy-acids of phosphorus and sulphur, sugars, amino-acids, amines, peptides, non-volatile organic acids and lower fatty acids. Both uni- and two-dimensional techniques were employed. In some instances, he demonstrated results obtained by high-voltage electrophoresis side by side with those obtained by low-voltage electrophoresis under equivalent conditions, showing a convincingly better resolution by the high-voltage technique.

Finally, Dr. Gross summarised the advantages and difficulties of the new technique and said that he was quite confident that there was a definite place for it in modern analytical chemistry, particularly for low-molecular compounds.

The Determination of Small Amounts of Iron

Some Aspects of New Colorimetric Methods*

By H. J. CLULEY

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Three colour reactions that seemed to merit particular attention have been examined. Certain interferences in the determination of ferrous iron with 4,7-diphenyl-1,10-phenanthroline have been overcome. Some interferences in the determination of ferrous iron with *syn*-phenyl 2-pyridyl ketoxime are reported, and the application of this reagent to the determination of iron in glass sand has been confirmed. The colour reaction between ferric iron, ethylenediaminetetra-acetic acid and hydrogen peroxide is recommended for determining larger amounts of iron, particularly in copper and its alloys.

It is not easy to select suitable methods for determining small amounts of iron from the many available in the literature. This paper deals with three methods, each of which appears to offer some distinct advantage.

Of the established reagents, 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) is probably the most outstanding on the basis of sensitivity and selectivity.

A newer reagent, *syn*-phenyl 2-pyridyl ketoxime, has the distinctive property of giving a sensitive colour reaction with ferrous iron in highly alkaline solutions. It can be used, for example, to determine iron in alkali-metal hydroxides and in the melts resulting from alkaline fusions of silicates.

Ferric iron reacts with ethylenediaminetetra-acetic acid and hydrogen peroxide in alkaline solution to produce a colour. This provides a method for determining iron that, although not particularly sensitive, is capable of yielding accurate results. It is our opinion that this reaction has not received the attention it deserves.

DETERMINATION OF IRON WITH BATHOPHENANTHROLINE

Bathophenanthroline reacts with ferrous iron within the pH range 2 to 9 to form the bright red *tris*-(4,7-diphenyl-1,10-phenanthroline) - ferrous ion, which has a maximum light absorption at 533 mμ. It can be extracted as an ion-association system with a suitable anion into any one of several organic solvents. The molar extinction coefficient of the complex is about 22,000, the exact value depending on the solvent used. This is approximately twice the value of the molar extinction coefficient of the familiar ferrous - 1,10-phenanthroline complex in aqueous solution. Moreover, the ease of extraction of the ferrous - bathophenanthroline complex confers two extra advantages over the use of 1,10-phenanthroline. Concentration of the iron complex into an organic solvent of smaller volume than that of the test solution results in an effective increase in the sensitivity of the test. Also, the blank values can be reduced by treating certain of the reagents used in the test procedure with bathophenanthroline, followed by extraction with an organic solvent to remove any iron.

Bathophenanthroline was first introduced as a reagent for iron by Smith, McCurdy and Diehl,[1] who used it for determining iron in water. It has since been used for determining serum iron[2,3] and for determining traces of iron in tungsten,[4,5] uranium-bearing materials,[5,6] copper,[7] bismuth,[8] germanium and its dioxide,[9] petroleum oils,[10] vanadium, chromium, titanium, niobium and tantalum metals, their alloys and compounds[5] and sexavalent molybdenum compounds.[11] It has also been used for determining ferrous iron in the presence of ferric iron[12,13] and for the determinations of corticoids,[14] hydrogen peroxide[15] and tocopherols,[16] all of which depend on the use of the reagent for determining ferrous in the presence of ferric iron.

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* Presented in part at the meeting of the Society on Thursday, February 8th, 1962.

Various workers have reported interferences in the determination of iron with bathophenanthroline. Smith, McCurdy and Diehl reported the formation of a yellow complex with cobalt,[1] but this is not extracted into organic solvents. Cuprous copper also gave a yellow colour in neutral or alkaline solutions, but below pH 7 a colourless complex formed. Relatively large amounts of these metals would therefore cause considerable interference by competing with the iron for the reagent. Zinc and nickel have also been reported to interfere similarly and to cause low results when there is insufficient bathophenanthroline to react with the iron *plus* the interfering metal.[9] Ammonium, alkali and alkaline-earth metal ions cause no interference, nor do Be^{2+}, Hg_2^{2+}, Al^{3+}, Ce^{3+}, Pb^{2+}, Cr^{3+}, UO_2^{2+} and Mn^{2+} ions when present in at least 10-fold excess over the amount of iron. It is apparent from the applications of bathophenanthroline that many other metal ions can be tolerated.

The anions Cl^-, I^-, NO_3^-, SO_4^{2-}, ClO_4^-, $S_2O_3^{2-}$, SCN^- and acetate do not interfere. Cyanide interferes by reacting with the bathophenanthroline - ferrous complex to produce the violet dicyano-*bis*-(4,7-diphenyl-1,10-phenanthroline) - ferrous complex. It is possible that this reaction could be used for determining traces of cyanide.

There have been reports that the formation of the iron - bathophenanthroline complex is hindered by phosphate,[4,9] and that low results are obtained in the presence of oxalate,[4] citrate[4,9] and tartrate.[11] We also observed serious interference from lactate, fluoride and pyrophosphate, traces of which caused extremely low recoveries of iron.

The main objective of the work reported here was to overcome the interference caused by these anions.

EXPERIMENTAL

The usual procedure for determining iron with bathophenanthroline is to add the reagent to the acetate-buffered test solution of pH 4 to 6, containing iron that has been reduced to the ferrous state; hydroxyammonium chloride is the most common reductant. After the colour has developed the complex can be extracted into one of several solvents. We prefer to use chloroform because it is denser than the aqueous phase. The chloroform extracts are diluted to a standard volume with absolute industrial ethanol, and the optical density of the solution is measured at 533 mμ. Modifications of this general procedure, designed to overcome interference by tartrate[11] and citrate,[4,5] have been described, but no method has been described in the literature for eliminating interference from lactate, oxalate, fluoride, phosphate or pyrophosphate.

Galliford and Newman[11] complexed sexavalent molybdenum with tartrate to prevent precipitation of the molybdenum during determinations of iron with bathophenanthroline, but this raised the problem of the slightly low results that tartrate caused. These low results arise because tartrate hinders the extraction of the complex in some way. It is widely known that the ferrous - 1,10-phenanthroline complex can be extracted into the polar solvent nitrobenzene by adding perchlorate ion to the test solution. The ion-association system formed between the ferrous - phenanthroline complex and perchlorate ion is reasonably soluble in nitrobenzene. By analogy, Galliford and Newman added perchlorate to the test solutions of molybdates containing tartrate. In this way they increased the solubility of the bathophenanthroline - ferrous complex in chloroform, and so overcame the interference from tartrate.

We have applied a similar procedure to the determination of iron in molybdates, sodium tungstate, antimony trichloride and tartaric acid. Tartrate was used to prevent precipitation in tests on the molybdates, tungstate and antimony trichloride. This procedure is referred to below as the "tartrate method." We could not overcome the rather more serious interferences caused by citrate, lactate, oxalate, fluoride, phosphate and pyrophosphate simply by adding perchlorate to the test solution. The use of ascorbic acid, which is a more powerful reducing agent than hydroxyammonium chloride, caused a slight improvement in the results, but they were still extremely low. We presumed that these anions were forming complexes with the ferric ions and so preventing their reduction to the ferrous state. The formation of such complexes might be expected to be considerably reduced in fairly concentrated acid solution, and we decided, therefore, to reduce the ferric iron to ferrous under rather acid conditions. The test solutions were adjusted to 1 or 2 M in perchloric acid before adding ascorbic acid to reduce the iron. The bathophenanthroline was then added to the solution, the pH was raised to between 4 and 6, and the determination was completed in the usual way. The results obtained by this method were encouraging. The method is referred to below as the "acid reduction method."

The results obtained by these procedures are shown in Tables I, II, III and IV, and comparison is made with results obtained by other methods, all of which involved a final colorimetric determination of ferric iron by a thiocyanate method. The only serious discrepancy is in the results obtained on sodium pyrophosphate. Details are also given of recovery experiments carried out with known amounts of added iron.

TABLE I

RESULTS OBTAINED BY THE TARTRATE METHOD

A 1-g sample was used for each test

Sample	Iron found by— tartrate method, %	Iron found by— thiocyanate method, %
Antimony trichloride	0·0012	0·0013
Molybdic acid	0·00021	0·0002
Sodium molybdate	0·00017	0·0002
Tartaric acid	0·00018	0·0002

TABLE II

RESULTS OF RECOVERY EXPERIMENTS BY THE TARTRATE METHOD

A 1-g sample was used for each test

Sample	Iron added, µg	Iron found, µg	Recovery, µg
Antimony trichloride	0	12	
	10	21	9
Molybdic acid	0	2·0, 2·1	
	10	12·1, 12·3	10·1
Sodium molybdate	0	1·7, 1·6	
	10	11·9, 11·6	10·1
Sodium tungstate	0	0 0	
	10	10·0, 9·8	9·9
Tartaric acid	0	1·8	
	5	6·8	5·0
	10	11·7	9·9

TABLE III

RESULTS OBTAINED BY THE ACID REDUCTION METHOD

Sample	Weight taken for acid reduction method, g	Iron found by— acid reduction method, %	Iron found by— thiocyanate method, %
Citric acid	1	0·00064	0·0008
Lactic acid	2·5	0·00027	0·0003
Oxalic acid	1	0·00035	0·0003
Orthophosphoric acid	1	0·00072	0·0008
Sodium fluoride	0·5	0·0022	0·002
Sodium oxalate	0·5	0·00080	0·0006
Sodium pyrophosphate	0·1	0·0010	Not detected

In each instance the bathophenanthroline method was a considerable improvement on the thiocyanate method. Most of the procedures ending with a thiocyanate determination were tedious; for example, the tests on the organic materials required a preliminary destruction of the organic matter.

METHODS

REAGENTS—

All reagents should be of analytical grade when appropriate.

Ammonia solution, approximately 5 N.

Ascorbic acid, B.P.

Ethanol—Industrial methylated spirit, 74° O.P.

TABLE IV

RESULTS OF RECOVERY EXPERIMENTS BY THE ACID REDUCTION METHOD

The weights of sample used are as shown in Table III

Sample	Iron added, µg	Iron found, µg	Recovery, µg
Citric acid	0	6·4	
	5	11·4	5·0
	10	16·5	10·1
Lactic acid*	0	9·9	
	5	14·9	5·0
	10	20·0	10·1
Oxalic acid	0	3·5	
	5	8·4	4·9
	10	13·5	10·0
Orthophosphoric acid	0	7·2	
	5	12·3	5·1
	10	17·3	10·1
Sodium fluoride	0	11·1	
	5	15·7	4·6
	10	21·2	10·1
Sodium oxalate*	0	5·8	
	5	10·7	4·9
	10	15·4	9·6
Sodium pyrophosphate	0	1·0	
	5	6·3	5·3
	10	10·7	9·7

* Different batch from that used in Table III.

Perchloric acid, 60 per cent. w/w, sp.gr. 1·54—This is approximately 10 M.

Potassium sodium tartrate.

Sodium acetate, hydrated.

Sodium hydrogen carbonate.

Bathophenanthroline solution, 0·001 M—Dissolve 0·0332 g of 4,7-diphenyl-1,10-phenanthroline in 100 ml of ethanol.

Chloroform.

Hydrochloric acid, approximately 5 N.

Hydroxyammonium chloride - ammonium perchlorate solution—Dissolve 50 g of hydroxyammonium chloride and 160 g of ammonium perchlorate in sufficient water to produce 1 litre; adjust the pH to between 3 and 4, if necessary, by adding ammonia solution. Transfer the solution to a large separating funnel, add 10 ml of bathophenanthroline solution and set aside for 15 minutes. Extract any pink colour by shaking vigorously with successive 10-ml portions of chloroform until the extracts are colourless. Discard the extracts, and store the solution in an iron-free glass or polythene bottle.

Sodium hydroxide solution, approximately 5 N.

Tartrate solution—Dissolve 63 g of tartaric acid in sufficient water to produce 1 litre. Add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution, adjust the pH to between 3 and 4 with sodium hydroxide solution, and transfer to a large separating funnel. Purify and store the solution as described for the hydroxyammonium chloride - ammonium perchlorate solution.

Standard iron solution—Dissolve 8·65 g of ammonium ferric sulphate in 50 ml of nitric acid, sp.gr. 1·42, and sufficient water to produce 1 litre.

$$1 \text{ ml} \equiv 1 \text{ mg of iron.}$$

Dilute standard iron solution—Dilute 10 ml of the standard iron solution to 1 litre with water; this solution must be freshly prepared before use.

$$1 \text{ ml} \equiv 10\ \mu\text{g of iron.}$$

PREPARATION OF CALIBRATION GRAPH—

By pipette or burette place amounts of dilute standard iron solution to cover the range 0 to 40 μg of iron in separate 150-ml separating funnels, and treat each aliquot as described below. Dilute with 20 ml of water, add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution and 10 ml of bathophenanthroline solution, and set aside for 15 minutes. Add 5 ml of chloroform, insert the stopper, shake the funnel vigorously for 30 seconds, and allow the layers to separate. Run the chloroform layer through a glass-wool plug supported in a small funnel into a 25-ml calibrated flask. Add another 5 ml of chloroform to the contents of the separating funnel, extract by shaking for a few seconds, allow the layers to separate, and add this extract to the first.

Dilute the combined extracts to the mark with ethanol, mix, and measure the optical density at 533 mμ in a 1-cm cell against the solution containing no added iron. Plot a graph relating optical density to amount of iron present (in micrograms); the graph should be linear and pass through the origin.

Note that an identical calibration graph can be prepared with ascorbic acid as reducing agent in place of the hydroxyammonium chloride. Calibration graphs prepared in accordance with the various procedures described below were identical.

PROCEDURES -

For sodium molybdate and sodium tungstate—Dissolve 1 g of sample in 40 ml of tartrate solution in a 150-ml separating funnel. Add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution, and adjust the pH of the solution to about 4 with hydrochloric acid or ammonia solution. Add 10 ml of bathophenanthroline solution, mix, and set aside for 15 minutes. Add 5 ml of chloroform, and complete the determination as described under "Preparation of Calibration Graph"; use as blank a solution prepared from the same amounts of reagents.

For molybdic acid—Dissolve 1 g of sample in approximately 10 ml of water and sufficient sodium hydroxide solution to give a pH of about 10·5, boil for a few minutes, and then cool. Transfer the solution to a 150-ml separating funnel, add 40 ml of tartrate solution, and complete the determination as described above for sodium molybdate and sodium tungstate.

For tartaric acid—Dissolve 1 g in 25 ml of water, and add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution and sufficient ammonia or sodium hydroxide solution to give a pH between 4 and 6. Add 10 ml of bathophenanthroline solution, and set aside for 15 minutes. Add 5 ml of chloroform, and complete the determination as described above.

The iron content of tartaric acid can also be determined by the acid reduction method with ascorbic acid, as described below.

For antimony trichloride—Dissolve 1 g of sample in a mixture of 4 ml of hydrochloric acid solution and 0·5 ml of perchloric acid. Add this solution slowly, with stirring, to a solution of 5 g of potassium sodium tartrate in 40 ml of water. Add 0·1 g of ascorbic acid and 10 ml of bathophenanthroline solution. Add sodium hydrogen carbonate in small portions until the pH is about 7. Warm the solution until it is clear, cool, and adjust the pH again if necessary to about 7 by adding sodium hydrogen carbonate. Heat the solution on a steam-bath for 15 minutes, cool, and transfer to a 150-ml separating funnel. Add 5 ml of chloroform, and complete the determination as described above.

If sodium hydroxide solution is used to neutralise the acid solution, a temporary region of high pH may occur in the solution, causing precipitation of the antimony; once precipitated, the antimony does not readily redissolve. Neutralisation with sodium hydrogen carbonate avoids this difficulty.

ACID REDUCTION METHOD—

(This method was applied to the determination of iron in citric, lactic, oxalic and phosphoric acids, sodium fluoride, sodium oxalate and sodium pyrophosphate. The weights used of each sample are shown in Table III.)

Dissolve the sample in sufficient water and perchloric acid to give a clear solution between 1 and 2 M in perchloric acid. Add 5 ml of freshly prepared 10 per cent. ascorbic acid solution and 10 ml of bathophenanthroline solution. Add 0·5 g of sodium acetate, raise the pH of the solution to between 4 and 6 by adding ammonia solution, and set aside for 20 minutes.

(for oxalates it is also advisable to heat the solution on a steam-bath for 5 minutes). Transfer the solution to a 150-ml separating funnel, add 5 ml of chloroform, and complete the determination as described above.

OTHER APPLICATIONS OF BATHOPHENANTHROLINE

We also use the acid reduction method for the routine determination of iron in lead, cerium, thorium, chromium and uranium compounds.

When ethanolic bathophenanthroline solution is added to aqueous test solutions a turbidity is often produced that makes visual estimations of traces of iron impossible. However, while determining iron in ascorbic acid we noticed that, when not less than 0·5 g of ascorbic acid was present, the test solution remained clear on addition of the bathophenanthroline solution. A simple test for the visual estimation of iron was developed, as described below.

> Transfer the solution containing iron to a 50-ml Nessler cylinder, and add 0·5 g of ascorbic acid. Adjust the pH of the solution to between 4 and 6, and add 4 ml of bathophenanthroline solution. Dilute to 50 ml with water, and set aside for 15 minutes. Compare any red colour produced with similarly prepared standards containing known amounts of iron.

The minimum amount of iron that can be detected by this procedure is about 0·1 μg.

DETERMINATION OF IRON WITH *syn*-PHENYL 2-PYRIDYL KETOXIME

Phenyl 2-pyridyl ketoxime (PPK) forms coloured water-soluble compounds with several transition-metal ions in neutral and alkaline solution, most of which can be extracted into organic solvents. Red derivatives are produced with both ferrous and ferric iron, but the lowest concentration of ferric iron that can be detected in solution with this reagent is about 5 p.p.m., whereas only 0·1 p.p.m. of ferrous iron produces a detectable colour. Phenyl 2-pyridyl ketoxime is applied, therefore, to the determination and detection of iron in the ferrous state.[17]

The ferrous - PPK complex contains three molecules of the oxime and one atom of iron. It is water soluble and has a wavelength of maximum absorption at 545 mμ. It is formed in neutral and highly basic solutions, and from solutions more than molar in sodium hydroxide it can be extracted into pentanol or chloroform. In the presence of a little ethanol, one extraction with pentanol serves to remove the iron complex from the aqueous phase, and the pentanol solution of the complex exhibits maximum absorption at 550 mμ.

Aqueous solutions of the iron complex are said to be unstable, whereas solutions in pentanol are stable and show a negligible diminution in absorption after standing for 24 hours.[17] Solutions of the iron compound in chloroform are light sensitive, but we found that they could be stabilised by adding ethanol. The solution of the iron complex in the chloroform - ethanol mixture had an absorption maximum at 550 mμ. Two extractions with chloroform are needed to remove the iron complex completely from the aqueous phase; on the other hand, chloroform has an advantage over pentanol in being denser than the aqueous phase. The practical work described below was carried out by the chloroform extraction method, and most of the results were compared with those obtained via extraction with pentanol; the results by both procedures were identical. The molar extinction coefficient of the complex is about 15,000 in both solvents, and Beer's law is obeyed at least over the range 0 to 2·8 μg of iron per ml.

The advantage of phenyl 2-pyridyl ketoxime is its colour reaction with ferrous iron in strongly alkaline conditions. It has been used by Trusell and Diehl[17] for determining iron in lithium, sodium and potassium hydroxides and in sodium carbonate. The time required for full development of the iron colour depends on the concentration of hydroxide. In fairly concentrated sodium hydroxide solutions the colour development is slow, but it can be hastened by heating the solution.

The measurement of the colour could be made on the aqueous solution, but there are two complications; as mentioned above the aqueous solutions of the complex are not particularly stable and the colour intensity depends on the alkalinity. The molar extinction coefficient of the iron - PPK complex is a maximum in 3 M sodium hydroxide, but decreases fairly rapidly in concentrations of sodium hydroxide on both sides of this value. Therefore, unless strict control of alkalinity is feasible it is recommended that the extraction procedure should be adopted.

To reduce iron to the ferrous state in these strongly alkaline conditions it is necessary to use sodium dithionite. This chemical usually contains appreciable amounts of iron, so that solutions prepared from it must be made free from iron before use. The solution is not stable under normal conditions and must be prepared freshly before use. We attempted to use several other reducing agents, but without success.

METHOD

The method is based on that of Trusell and Diehl.[17]

REAGENTS—

Chloroform.

Ethanol—Industrial methylated spirit, 74° O.P.

Phenyl 2-pyridyl ketoxime solution, 0·2 per cent.—Dissolve 2 g of *syn*-phenyl 2-pyridyl ketoxime in 1 litre of 0·1 M hydrochloric acid.

Sodium dithionite solution, 10 per cent. (iron-free)—Dissolve 2 g of sodium dithionite in 10 ml of water, add 5 ml of phenyl 2-pyridyl ketoxime solution and 5 ml of 10 M sodium hydroxide, and set aside for 10 minutes. Extract the iron - PPK complex into 10 ml of chloroform and 5 ml of ethanol, and discard the extract.

Sodium hydroxide solution, 10 M (iron-free)—Dissolve 400 g of sodium hydroxide in 800 ml of water, add 10 ml of sodium dithionite solution and 50 ml of PPK solution, and set aside for 10 minutes. Extract the iron - PPK complex into 100 ml of pentanol and 25 ml of ethanol, and discard the extract. Dilute to 1 litre with water, and store in a polythene bottle.

Standard iron and dilute standard iron solutions—Prepare as described under the procedures for the determination of iron with bathophenanthroline, p. 6.

PREPARATION OF CALIBRATION GRAPH—

By pipette or burette, place amounts of dilute standard iron solution to cover the range 0 to 70 µg of iron in separate 50-ml beakers, and treat each aliquot as described below. Dilute with water to produce 10 ml, add 25 ml of 10 M sodium hydroxide, 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Add 5 ml of ethanol and 5 ml of chloroform, extract by shaking vigorously for 30 seconds, and then allow the layers to separate. Run the chloroform layer through a glass-wool plug supported in a small funnel into a 25-ml calibrated flask. Add another 5 ml of chloroform to the contents of the separating funnel, and extract and separate as before.

Dilute the combined extracts to the mark with ethanol, mix, and measure the optical density at 550 mµ in a 1-cm cell against the solution containing no added iron. Plot a graph relating optical density to amount of iron present (in micrograms); the graph should be linear and pass through the origin.

Note that an identical calibration graph can be prepared with pentanol as extractant in place of the chloroform. In this instance extract once with 10 ml of pentanol and dilute the extract to 25 ml with pentanol.

PROCEDURE—

Dilute the solution of the sample with water or sodium hydroxide solution to a volume of 35 ml, the concentration of sodium hydroxide being about 7 M. Add 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Complete the determination as described under "Preparation of Calibration Graph"; use as blank a solution prepared from the same amounts of the reagents used.

INTERFERENCES—

Recovery experiments were carried out by the procedure described above on 40-µg amounts of iron in the presence of various salts. The weights used and the results obtained are shown in Table V.

TABLE V

RECOVERY OF 40-μg AMOUNTS OF IRON IN THE PRESENCE OF VARIOUS SALTS

Salt added	Weight of salt taken, g	Recovery of iron, μg
Sodium acetate	1	40·4
Sodium arsenate	1	Heavy precipitate
	0·1	36·5*
Sodium chloride	1	40·1
Trisodium citrate	1	39·7
Sodium fluoride	0·002	40·1
Sodium molybdate	1	32·7
	0·5	35·9
Sodium nitrate	1	40·0
Sodium oxalate	0·1	31·0*
Ammonium perchlorate	1	39·8
Disodium hydrogen orthophosphate	1	40·1*
Sodium sulphate	1	39·0
Potassium sodium tartrate	1	27·9
	0·5	35·6
Sodium tetraborate	1	40·1

* A precipitate or turbidity formed during the test, and the iron was extracted from the filtered test solution.

Recovery experiments with 40-μg amounts of iron were also carried out in the presence of some other transition-metal cations known to give colour reactions with PPK. The results are shown in Table VI.

TABLE VI

RECOVERY OF 40-μg AMOUNTS OF IRON IN THE PRESENCE OF OTHER TRANSITION METALS

Ion added	Weight of ion added, μg	Recovery of iron, μg
Co^{2+}	40	38·1
Cu^{2+}	40	36·5
Mn^{2+}	40	38·1
Ni^{2+}	40	36·1
Pt^{2+}	100	38·4

Quantitative recoveries of 40-μg amounts of iron were obtained in the presence of 10-mg amounts of cerium^{III} and mercury^{I} and 5-mg amounts of aluminium, calcium and zinc. However, mercury gave a black precipitate and calcium a white precipitate, and it was necessary to extract with pentanol from solutions containing these metals (because pentanol has a lower density than the aqueous phase) in order to obtain good recoveries.

Precipitates were also given by cadmium (yellow), lead (black) and magnesium (white), and low results were obtained in the presence of 5 mg of cadmium and 10 mg each of the other two metals. The interference caused by magnesium was particularly serious.

DETERMINATION OF IRON IN GLASS SAND

Trusell and Diehl[17] described the application of PPK to the determination of iron in glass sand. The sample was fused in a platinum vessel with a mixture of equimolecular amounts of sodium carbonate and sodium tetraborate. When the melt was cool it was dissolved in water, treated with sodium dithionite and PPK solution, and the determination was completed in the usual way. They obtained excellent results on NBS glass sand.

In our hands this method gave low and variable results. It was thought that dissolution of the carbonate - borate melt in water gave a solution insufficiently alkaline to permit quantitative extraction of the ferrous - PPK complex. A further trial of the method was made in which sodium hydroxide solution was added to the solution of the melt in water; development and extraction of the colour then gave quantitative recovery of the iron. The method, with this minor modification, is quick, simple and accurate. The advantage over conventional methods is that removal of the silica (with hydrofluoric acid) is unnecessary.

REAGENTS—

Fusion mixture—An equimolecular mixture of anhydrous sodium carbonate and sodium tetraborate.

The other reagents required are those listed on p. 9.

PROCEDURE—

Weigh 0·1 g of the finely ground sand into a platinum dish previously rendered free from iron. Add 1 g of the fusion mixture, and blend it intimately with the sample. Place the dish in a muffle furnace at 1000° C for 10 minutes. Allow the melt to cool, and dissolve it by warming with 10 ml of water. Transfer the solution to a small beaker with 25 ml of 10 M sodium hydroxide, and add 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Complete the determination as described under "Preparation of Calibration Graph," p. 9; use for the blank test 1 g of the fusion mixture and the same amounts of reagents as used in the test.

RESULTS—

The results obtained by this procedure were compared with those obtained by the method described in British Standard 2975:1958, "Sand for making colourless glasses." In the B.S. method the silica is removed by the conventional hydrofluoric acid and sulphuric acid fuming procedure, and the iron is determined in the residue with thioglycollic acid. The results in Table VII show close agreement between the two methods.

The PPK method is appreciably quicker, as no evaporation stage is involved. A further advantage of the PPK method is that it is unnecessary to grind the sand to the extremely fine state essential in conventional methods for achieving ready solubility of the sand in hydrofluoric acid.

TABLE VII

DETERMINATION OF FERRIC OXIDE CONTENT OF GLASS SAND BY THE BRITISH STANDARD AND PHENYL 2-PYRIDYL KETOXIME METHODS

	Ferric oxide found by—	
	B.S. method, %	PPK method, %
Sample A	0·034	0·034
	0·034	0·035
Sample B	0·076	0·077
	0·076	0·076

DETERMINATION OF IRON BY COLOUR REACTION WITH EDTA AND HYDROGEN PEROXIDE

Metals that form coloured cations usually give more intense colours in the form of their EDTA complexes. For this reason EDTA and related complex-forming compounds have occasionally been suggested as reagents for the colorimetric determination of certain metals, but such procedures obviously have little selectivity. However, Cheng and Lott[18] observed that addition of hydrogen peroxide to an ammoniacal solution containing ferric iron and EDTA resulted in the formation of a distinctive purple colour. Only cobalt (blue colour) was found to react similarly, though metals forming peroxide complexes (not EDTA - peroxide complexes), such as chromium, molybdenum, uranium and vanadium, could interfere. These workers later advocated the use of the ferric - EDTA - peroxide colour for the colorimetric determination of iron in limestone and clay.[19]

The Fe - EDTA - H_2O_2 colour was also subsequently investigated by Ringbom, Siitonen and Saxén.[20] These workers concluded that the purple colour was due to a peroxy derivative of the ferric - EDTA complex, but the blue colour formed under similar conditions by cobalt was attributed to the cobalt^{III} - EDTA complex, the peroxide serving merely to oxidise the cobalt to the higher valency state.

Ringbom *et al.* pointed out that the Fe - EDTA - H_2O_2 complex could be used for the colorimetric determination of iron even in the presence of metals forming coloured EDTA (but not EDTA - peroxide) complexes. This was achieved by suitable treatment of a sample aliquot with EDTA, ammonia and peroxide to develop the iron colour, and by using as reference solution a further aliquot of the sample solution to which only EDTA and ammonia were added; the difference in optical densities was that due to the Fe - EDTA - H_2O_2 complex. Ringbom *et al.* further observed that the copper - EDTA complex had a favourably low absorption at wavelengths at which the iron colour could be measured, so that determination of iron in the presence of substantial amounts of copper appeared practicable. These workers quoted some results for the iron content of brass, directly determined in this manner, but gave little experimental detail.

The use of the Fe - EDTA - H_2O_2 complex for the colorimetric determination of iron has attracted little attention. However, the possible application in the presence of large amounts of copper has obvious merit, particularly as many reagents for iron, such as 1,10-phenanthroline and 2,2′-bipyridyl and their derivatives, suffer interference from copper. Some observations on the Fe - EDTA - H_2O_2 method and on its application to copper alloys are described below.

EXPERIMENTAL

ABSORPTION SPECTRA—

The procedure of Ringbom *et al.*[20] was used for developing the Fe - EDTA - H_2O_2 colour. To a solution containing 0·5 mg of ferric iron was added in turn 10 ml of 0·01 M EDTA (disodium salt), one drop of ammonia solution, sp.gr. 0·88, to make the solution alkaline, 2·0 ml of 100-volume hydrogen peroxide and 3·0 ml of ammonia solution; water was then added to give a final volume of 25 ml. These conditions were subsequently shown to give full development of the iron colour. A corresponding solution of the Fe - EDTA complex was prepared in the same manner, but omitting the hydrogen peroxide. To study the absorption spectrum of the copper - EDTA complex, a 25-ml solution containing 100 mg of copper (as sulphate) and an excess of EDTA, together with ammonia but no peroxide, was also prepared. The optical densities of these three solutions were measured over the range 400 to 600 mμ in 4-cm cells with water as the reference.

The absorption spectra plotted from these results are shown in Fig. 1. The Fe - EDTA - H_2O_2 complex shows a broad absorption peak at about 500 mμ with maximum absorption

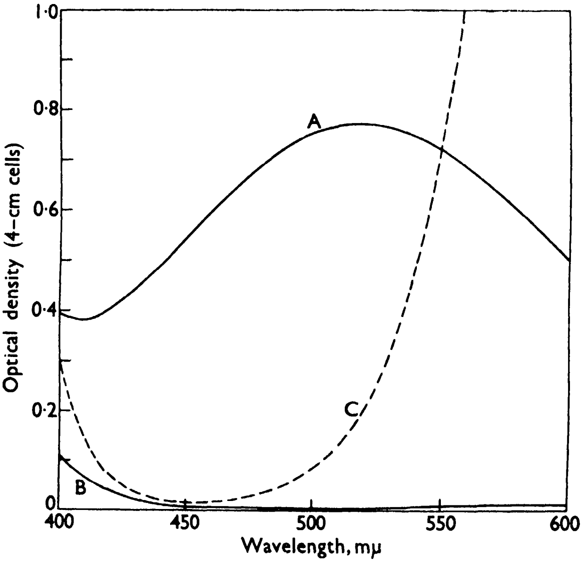

Fig. 1. Absorption spectra: curve A, 0·5 mg of iron, as the Fe - EDTA - H_2O_2 complex, in 25 ml; curve B, 0·5 mg of iron, as the Fe - EDTA complex, in 25 ml; curve C, 100 mg of copper, as the Cu - EDTA complex, in 25 ml

at 520 mμ, as reported by Cheng and Lott[18] and by Ringbom *et al.*[20] At 500 to 520 mμ, the Fe - EDTA complex has zero absorption. The curve obtained for the Cu - EDTA complex showed that, despite its intense colour at the concentration employed, the absorption is low in the region where the iron colour can be measured; as found by Ringbom *et al.*[20] the absorption is minimal at 450 mμ.

To determine iron in the presence of much copper by the use of a suitable reference solution to eliminate the absorption due to copper, the choice of wavelength lies between 450 mμ, at which absorption due to copper is least, and 520 mμ, at which absorption of the iron colour is greatest. With the solution containing 100 mg of copper, the optical densities measured against water were 0·01 at 450 mμ and 0·20 at 520 mμ; from this point of view measurement at 450 mμ, as suggested by Ringbom *et al.*, is preferable. On the other hand, the absorption of the iron complex at 450 mμ is only about two-thirds that at 520 mμ.

It appeared practicable to use either wavelength and, in fact, measurement at both wavelengths was made throughout the work described here.

EFFECT OF DIFFERENT PROPORTIONS OF REAGENTS—

Experiments were made with 0·5-mg amounts of iron in a final volume of 25 ml to ascertain the effect on the iron colour of different proportions of EDTA, peroxide and ammonia; the results are shown in Table VIII

Variation of the volume of ammonia solution, sp.gr. 0·88, from 1 to 5 ml, corresponding to final pH values of 10·7 to 11·3, had no effect on the colour; these and other experiments confirmed the conclusion[20] that the pH should be above 10·5 for full development of the colour. Restricting the volume of 0·01 M EDTA to 1 ml, *i.e.*, little greater than the amount equivalent to 0·5 mg of iron present, resulted in incomplete development of the colour, as assessed by measurement at 520 mμ. A minimum of 2 ml of the EDTA solution and 2 ml of the hydrogen peroxide appeared desirable, larger amounts having no effect on the colour. It thus appeared that the proportions of EDTA, hydrogen peroxide and ammonia could be varied appreciably, provided that the necessary minimum proportions were exceeded.

TABLE VIII

EFFECT OF DIFFERENT PROPORTIONS OF REAGENTS ON THE Fe - EDTA - H_2O_2 COLOUR

Each solution of final volume 25 ml contained 0·5 mg of iron; optical densities measured in 4-cm cells

Volume of 0·01 M EDTA, ml	Volume of 100-volume hydrogen peroxide, ml	Volume of ammonia solution, sp.gr. 0·88, ml	pH of final solution	Optical density at—	
				520 mμ	450 mμ
10	2	1	10·7	0·763	0·520
10	2	3	11·1	0·772	0·523
10	2	5	11·3	0·769	0·520
1	1	1	Not measured	0·710	0·536
10	1	1	Not measured	0·756	0·510
10	3	5	Not measured	0·771	0·523
1	2	3	Not measured	0·756	0·536
2	2	3	Not measured	0·768	0·521
4	2	3	Not measured	0·767	0·518

It is noteworthy that the two experiments in which only 1 ml of 0·01 M EDTA was used gave incomplete development of the colour as indicated by the optical density at 520 mμ, but at 450 mμ enhanced absorptions were observed. It would appear that under these conditions a different complex is being formed.

For calibration purposes, and in later experiments, the amounts of reagents used for development of the iron colour were 10 ml of 0·01 M EDTA, 2 ml of 100-volume hydrogen peroxide and 3 ml of ammonia solution, sp.gr. 0·88.

EFFECT OF TIME—

The formation of the Fe - EDTA - H_2O_2 colour appears to be virtually instantaneous, and the optical densities of solutions of the complex can be measured immediately after preparation. Re-measurement of the optical densities of two solutions after 1 hour's standing in artificial light gave the following results, the original optical densities being given in brackets: at 520 mμ, 0·640 (0·646) and 0·634 (0·632); at 450 mμ, 0·436 (0·434) and 0·432 (0·432).

The colours are therefore stable for at least an hour, but were observed to undergo appreciable fading overnight. The use of smaller amounts of hydrogen peroxide in development of the colour results in more rapid fading, the fading presumably being attributable to decomposition of the excess hydrogen peroxide in the alkaline solution; after it has faded the colour can be instantly regenerated by addition of peroxide.

CALIBRATION—

For calibration purposes colours were developed for 0·1- to 0·5-mg amounts of iron and measured against appropriate reference solutions, *i.e.*, containing the same amounts of iron and other reagents, but no peroxide. The results are shown in Table IX. Beer's law is obeyed for measurement both at 520 mμ and at 450 mμ, but, as observed earlier, measurement at the shorter wavelength results in a loss of sensitivity of about one third.

TABLE IX

CALIBRATION RESULTS

Final volume of solution 25 ml; optical densities measured in 4-cm cells

Iron present, mg	Optical density at 520 mμ	Optical density per mg of iron	Optical density at 450 mμ	Optical density per mg of iron
0·1	0·154	1·54	0·103	1·03
0·2	0·306	1·53	0·206	1·03
0·3	0·461	1·54	0·311	1·04
0·4	0·614	1·54	0·413	1·03
0·5	0·772	1·54	0·523	1·05
		Mean 1·54		Mean 1·04

EFFECT OF COBALT—

From the earlier published work it was apparent that cobalt was the element most likely to interfere with the iron colour. Lott and Cheng[19] stated that the cobalt colour could be inhibited by cyanide, but gave no practical details. In this work a few experiments were made to assess the extent of interference by cobalt and to examine its inhibition by cyanide.

With a solution of 0·5 mg of cobalt, use of the colour development procedure adopted for iron gave a pure blue colour, which was not discharged on addition of cyanide. However, if the cyanide was added at an earlier stage, *i.e.*, after the addition of EDTA and a slight excess of ammonia to a cobalt solution, there resulted a pale yellow colour, presumably that of the cobalt - cyanide complex; subsequent addition of hydrogen peroxide and more ammonia caused no change in the colour. The formation of the blue cobalt colour can be thus inhibited if cyanide is added at the correct stage.

Some quantitative results on the interference of cobalt and its inhibition are shown in Table X; the optical densities quoted were measured against reference solutions containing neither iron nor cobalt, so that the total absorptions of the colours obtained could be observed. These results show that the colour due to cobalt, if not inhibited, gives an absorption equivalent to about $\frac{1}{4}$ and $\frac{1}{9}$ of the same weight of iron for measurements at 520 and 450 mμ, respectively. The yellow colour resulting from the complexing of cobalt with cyanide was found to give zero absorption at 520 mμ, but a small absorption at 450 mμ; the latter could be effectively eliminated by using as a reference solution an aliquot of the cobalt-containing sample solution treated with EDTA, ammonia and cyanide, but not peroxide. It was apparent that development of the Fe - EDTA - H_2O_2 colour was unaffected by the presence of cyanide.

TABLE X

INTERFERENCE OF COBALT AND ITS INHIBITION BY CYANIDE

Iron present, mg	Cobalt present, mg	Volume of 10 per cent. potassium cyanide added, ml	Optical density at— 520 mμ	Optical density at— 450 mμ
0	0·5	0	0·180	0·060
0	0·5	1	0	0·041
0·5	0	0	0·772	0·523
0·5	0	1	0·768	0·515
0·5	0·5	1	0·774	0·562

MEASUREMENT OF THE IRON COLOUR IN THE PRESENCE OF MUCH COPPER—

Attention was then directed to the specific application of determining iron in the presence of a large amount of copper. To assess the accuracy of measurement of the iron colour in this context, solutions containing 100 mg of copper (as sulphate) and various amounts of iron were prepared in duplicate. In one solution of each pair, the iron colour was developed with EDTA, hydrogen peroxide and ammonia as previously described, except that the amount of EDTA used was increased to 12 ml of a 0·15 M solution to cater for the large amount of copper present. The other solution of each pair was treated similarly, except that no peroxide was added, to serve as the reference solution for the spectrophotometric measurements.

The results obtained, corrected for the small amount of iron found in the copper sulphate and other reagents, are shown in Table XI. The amounts of iron deduced from measurements at either wavelength are of acceptable accuracy, and show that as little as 0·05 mg of iron can readily be measured in the presence of 100 mg of copper, *i.e.*, equivalent to 0·05 per cent. of iron in copper.

TABLE XI

MEASUREMENT OF IRON COLOURS IN THE PRESENCE OF 100 mg OF COPPER

	Measurement at 520 $m\mu$		Measurement at 450 $m\mu$	
Iron added, mg	Optical density corrected for blank	Iron found, mg	Optical density corrected for for blank	Iron found, mg
0·055	0·086	0·056	0·057	0·055
0·145	0·220	0·143	0·151	0·145
0·300	0·456	0·296	0·313	0·302

Subsequently experiments were carried out to apply the procedure to the determination of iron in copper alloys. For this purpose alloys were dissolved in hydrochloric and nitric acids, tartaric acid then being added to prevent precipitation of tin when the colour was subsequently developed in ammoniacal solution. It was found desirable to restrict the amount of acid used for solution of the alloys, otherwise the amount of ammonium salts produced on neutralisation tended to buffer the solution to a pH unfavourably low for development of the iron colour. The procedure adopted was found to give a pH of about 10·7 at the final colour stage, *i.e.*, adequate for full development of the iron colour, as shown earlier.

Details of the full method evolved for the direct determination of iron in copper alloys are given below.

METHOD

REAGENTS—

Standard iron solution—Dissolve 0·1000 g of pure iron in 4 ml of diluted nitric acid (1 + 1), and dilute with water to 1 litre.

1 ml ≡ 0·1 mg of iron.

EDTA solution, approximately 0·01 M—Dissolve 3·7 g of disodium ethylenediaminetetraacetate dihydrate in sufficient water to produce 1 litre.

EDTA solution, approximately 0·15 M—Dissolve 56 g of disodium ethylenediaminetetraacetate dihydrate in sufficient water to produce 1 litre.

Ammonia solution, sp.gr. 0·88.

Hydrogen peroxide, 100 *volume.*

Hydrochloric acid, sp.gr. 1·18.

Nitric acid—Dilute nitric acid, sp.gr. 1·42, with an equal volume of water.

Tartaric acid solution, 10 *per cent., aqueous.*

PREPARATION OF CALIBRATION GRAPH—

In each of two 25-ml calibrated flasks place, by pipette, 1-ml portions of standard iron solution (1 ml ≡ 0·1 mg of iron). To each add 10 ml of 0·01 M EDTA and one drop of ammonia solution. To the contents of one flask add 2 ml of hydrogen peroxide; to the contents of both flasks add 3 ml of ammonia solution, dilute to the mark with water, and mix. Measure the optical density of the iron colour in 4-cm cells at 520 $m\mu$ and at 450 $m\mu$; use as reference the solution containing no peroxide.

Repeat the procedure with amounts of standard iron solution up to 5 ml and also in the absence of added iron. Correct the optical densities of the iron solutions for the small absorption found in the absence of added iron, and plot two calibration graphs (corresponding to the measurements at the two wavelengths) for the results. The graphs should be linear and pass through the origin.

PROCEDURE—

Weigh 1 g of sample, preferably in the form of drillings, into a 100-ml beaker (see Note 1). Add 5 ml of hydrochloric acid and 2·5 ml of diluted nitric acid (1 + 1), warm to initiate the reaction, and then remove from the source of heat. If necessary, heat again to complete the solution of the alloy, add 10 ml of water, and boil for 2 minutes to remove oxides of nitrogen. Cool, add 10 ml of tartaric acid solution, and dilute the solution to 50 ml in a calibrated flask.

By pipette, place 5-ml aliquots into each of two 25-ml calibrated flasks (see Note 2), and add to each 12 ml of 0·15 M EDTA and 1·0 ml of ammonia solution. To one solution add 2·0 ml of hydrogen peroxide, and to the other add 2 ml of water. Dilute each solution to the mark with ammonia solution, and mix.

Measure the optical density of the iron colour in 4-cm cells at 520 mμ and at 450 mμ; use as reference the solution to which no peroxide was added. Correct each reading for any blank value obtained by applying the procedure in the absence of a sample, and from the corrected optical densities deduce the iron content of the sample.

NOTES—

1. A 1-g sample is suitable for iron contents of 0·05 to 0·5 per cent.; for 0·5 to 1 per cent. of iron, use 0·5 g of sample.

2. After washing before use, shake the flasks free from superfluous water; if a millilitre or two of water remains in the flasks, the amount of ammonia required for the final dilution to 25 ml may be insufficient to give the pH necessary for full development of the iron colour.

RESULTS—

The procedure was applied to 1-g portions of pure copper, to which known amounts of iron were added after dissolution. The results (see Table XII) gave good agreement with the amounts of iron added, and showed that the method adopted for solution of samples, and in particular the use of tartaric acid, had no deleterious effect on the development of the iron colour.

TABLE XII

DETERMINATION OF KNOWN AMOUNTS OF IRON ADDED TO PURE COPPER

Weight of copper taken, g	Iron added, %	Iron found by measurement at— 520 mμ, %	450 mμ, %
1·0	0·05	0·049	0·048
1·0	0·15	0·150	0·148
1·0	0·30	0·296	0·299

The procedure was also applied in triplicate to two standard samples of copper alloy; these were a bronze (B.C.S. No. 207, 0·06 per cent. of iron) and a manganese brass (B.C.S. No. 179, 0·91 per cent. of iron) and the amounts of sample used were, respectively, 1·0 and 0·5 g. Further, to simulate samples with intermediate iron contents, iron was determined in three mixtures of the two standard alloys.

The results obtained are shown in Table XIII. On the standard samples close agreement was obtained between replicate determinations and between the values deduced from measurements at the two wavelengths; the latter observation provided strong evidence that interference from the constituents of the alloys was not occurring. The mean values obtained on the standard alloys were lower than the certified values, but fell within the range of results from which the certified values were obtained. The results on the mixtures were completely consistent with the iron contents calculated from the mean values found for the standard alloys.

It would appear that measurement at 520 or 450 mμ can be used with equal success for determining iron in copper alloys. For samples of an unfamiliar nature, measurement at both wavelengths may be a useful precaution in that interference, for example, from an unsuspected cobalt content, would be revealed by disparity between the results obtained at 520 and 450 mμ.

TABLE XIII

DETERMINATION OF IRON IN STANDARD SAMPLES OF COPPER ALLOYS

Sample	Weight of sample, g	Iron found by measurement at— 520 mμ, %		Iron found by measurement at— 450 mμ, %		Certified iron content and range of results, %	Calculated iron content (for mixtures), %
Bronze C, B.C.S. No. 207	1	0·052 0·053 0·052	mean 0·052	0·051 0·053 0·052	mean 0·052	0·06 (0·05 to 0·08)	—
Manganese brass B, B.C.S. No. 179	0·5	0·82 0·82 0·81	mean 0·82	0·83 0·82 0·82	mean 0·82	0·91 (0·81 to 0·98)	—
B.C.S. No. 207 B.C.S. No. 179	0·5 0·1	0·18		0·18		—	0·18
B.C.S. No. 207 B.C.S. No. 179	0·5 0·25	0·31		0·31		—	0·31
B.C.S. No. 207 B.C.S. No. 179	0·3 0·6	0·57		0·57		—	0·56

SUMMARY

The three colour reactions reviewed in this paper cover between them a wide field of application. The influences of diverse ions on iron determinations by each method are reported in more detail than previously, and practical procedures have been devised to overcome several interferences.

Bathophenanthroline is recommended for highly accurate determinations of iron in microgram amounts under most conditions. For determining iron in highly alkaline solutions, however, phenyl 2-pyridyl ketoxime gives a useful and reasonably sensitive colour reaction. For determining larger amounts of iron in the presence of many other metals, the colour reaction between ferric iron, EDTA and hydrogen peroxide is extremely useful. It can be highly recommended for determining iron in copper-bearing metals.

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A Scheme for the Quantitative Analysis of a Phosphate Rock including the Use of a Benzoate Separation

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In a scheme for analysing a phosphate rock, group-3 metals are separated by a double precipitation as benzoates at a pH of 3·5 and calcium is separated by a double precipitation as the oxalate at a pH of 4·0. Gravimetric finishes are employed for determining aluminium, calcium and magnesium. The determination of aluminium as the oxinate in the presence of phosphate, after the separation of interfering metals by a cupferron extraction, is examined. In the colorimetric determination of iron as the ferrous - bipyridyl complex, interference by phosphate is avoided by reduction in acid solution with ascorbic acid (in place of hydroxylamine hydrochloride) followed by formation of the complex in a chloroacetate buffer solution (pH 3·0). A colorimetric determination is employed for silica, in which fluoride and phosphate can be tolerated up to certain limits. The determination of other elements is discussed.

THE scheme proposed here for analysing a phosphate rock is divided into two main sections: (*i*) a systematic gravimetric analysis, with group separations for determining aluminium, calcium and magnesium, and (*ii*) individual determinations of the other elements by specific methods on separate portions of samples.

The chief difficulty in analysing phosphate rocks lies in the systematic analysis, as the separation of minor amounts of group-3 metals from the predominant alkaline earths by the ammonia precipitation is vitiated by the presence of major amounts of phosphate.

Various procedures have been devised to overcome the problem of chemical separations in the presence of phosphate; for example, Harvey[1] removed calcium by precipitation as sulphate in alcoholic solution and then precipitated the group-3 elements as phosphates; Hoffman and Lundell[2] avoided systematic analysis and determined each element separately.

As the neutral or weakly alkaline conditions of the ammonia separation cause the phosphates of the alkaline earths to co-precipitate with the group-3 hydroxides, it would appear that precipitation at a lower pH, by an alternative reagent, might effect a clean separation.

Benzoic acid, a possible reagent for this separation, was first introduced into chemical analysis by Kolthoff, Stenger and Moskovitz[3] who used it to separate titanium and the tervalent metals from the bivalent metals in weakly acid solutions. The work of Smales[4] and Jewsbury and Osborn[5] suggests that the former elements are completely precipitated from solutions having pH values between 3 and 4. Benzoic acid has also been proposed for use in a scheme of qualitative analysis in the presence of phosphate.[6]

The investigation described here was an attempt to establish conditions for the use of this reagent in quantitative separations and to develop a scheme for the main analysis of phosphate rocks.

Of the elements determined on separate samples of phosphate rock, the alkalis, manganese, titanium, phosphate, carbonate and water present no special difficulties. The gravimetric determination of silica in the presence of fluoride is tedious and not entirely satisfactory[2] and, on balance, the direct colorimetric procedure is to be preferred. The determination of iron may give rise to some problems, and the determination of fluoride remains difficult.

SYSTEMATIC ANALYSIS

The systematic scheme is based on the combination of separations listed below.

1. A double precipitation of the group-3 metals as benzoates at pH 3·5. Our work shows that this effectively separates these metals from calcium, magnesium and small amounts of manganese.

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2. As the benzoate precipitate contains an indeterminate amount of phosphate, simple ignition and weighing gives no useful information. Aluminium is therefore separated from phosphate and determined gravimetrically by an oxine precipitation, after removal of iron and titanium by a cupferron extraction. Chromium^{III} and uranium^{VI} interfere, as they are partly or completely precipitated as phosphates, benzoates and oxinates and are not removed by a cupferron extraction; however, they are only trace constituents of phosphate rocks of the apatite type.

3. The subsequent separation of calcium from magnesium and phosphate is by a double-acid oxalate precipitation as described by Hoffman and Lundell.[2]

pH AND COMPLETENESS OF PRECIPITATION WITH BENZOIC ACID—

Benzoate precipitations (described in the method given below) were made from solutions containing 25·0 mg per 100 ml of Al_2O_3, Fe_2O_3 or TiO_2 (the titanium solution was in N sulphuric acid), the pH being varied. Oxidising conditions were necessary for the precipitation of iron. After filtration the concentration of each metal remaining in solution was determined colorimetrically. The figures shown in Table I indicate that precipitation of these elements is virtually complete at pH 3·0 and above.

TABLE I

COMPLETENESS OF PRECIPITATION FROM SOLUTIONS OF DIFFERENT pH VALUES

Solutions containing 25·0 mg of the metal oxides

pH of precipitation	Concentration of the element remaining in solution		
	Aluminium as Al_2O_3, mg per 100 ml	Iron as Fe_2O_3, mg per 100 ml	Titanium as TiO_2, mg per 100 ml
2·0	0·22	0·37	0·15
2·5	0·09	0·10	0·03
3·0	0·03	0·02	0·01
3·5	0·01	0·02	0·01
4·0	0·01	0·02	0·01

CONTAMINATION OF THE BENZOATE PRECIPITATE—

Benzoate precipitations were carried out at various pH values on a synthetic solution containing the group-3 elements, alkaline earths and a small amount of manganese. These precipitates were ignited and examined spectrographically for calcium, magnesium and manganese. The results in Table II show that the contamination of the precipitate increases with pH and that a double precipitation at pH 3·5 effects a good separation of the group-3 elements from the bivalent metals.

TABLE II

CO-PRECIPITATION OF CALCIUM, MAGNESIUM AND MANGANESE

Precipitations from solutions containing 100·0 mg of CaO, 10·0 mg of MgO, 25·0 mg of Al_2O_3, 10·0 mg of Fe_2O_3, 2·5 mg of TiO_2, 0·10 mg of MnO and 75·0 mg of P_2O_5 per 100 ml of solution

Precipitation pH	Amount found in the ignited precipitates		
	Calcium oxide, %	Magnesium oxide, %	Manganese oxide, %
4·0	2·0	0·1	0·1
3·5	0·9	0·05	0·05
3·0	0·5	0·04	0·03
3·5 (double precipitation)	0·1	0·03	not detected

CHOICE OF pH FOR THE SEPARATION—

As the contamination of the benzoate precipitate increases with pH, the lowest pH at which the precipitation is complete (3·0) appears to be the most favourable. However, the final choice of pH was governed by the solubility of benzoic acid, which decreases with pH.

At the concentration used (1 g per 100 ml), benzoic acid comes out of solution at a pH of 3·0 at about 50° C and so interferes with the filtration; by working at a pH of 3·5 this difficulty is avoided.

Determination of Aluminium

According to Lundell and Knowles,[7] aluminium can be separated from phosphate by precipitation as the oxinate from ammoniacal solution. Our work shows that this separation is also satisfactory in acetic acid - acetate solution, provided that the precipitation is made by first adding the oxine to a N hydrochloric acid solution and then raising the pH to about 5 with ammonia and ammonium acetate. Under these conditions 25·0 mg of alumina could be correctly determined in the presence of 50·0 mg of phosphorus pentoxide. If the pH is adjusted to about 5 before adding oxine, aluminium phosphate is precipitated out and results are low; prolonged standing of the solution improves the recovery as the aluminium phosphate is slowly converted to the oxinate.

In the literature, various values are given for the excess of oxine required. A considerable excess was used by Stumpf[8] (about 10 per cent.) and Miller and Chalmers[9] (about 2 mg of excess per ml); as a result of our investigations, we are in agreement with the values they suggest.

METHOD

REAGENTS—

Benzoic acid—Analytical-reagent grade.

Ammonium benzoate wash solution—A solution containing 0·5 per cent. w/v of benzoic acid and 0·5 per cent. w/v of ammonium benzoate in water.

Ammonium acetate solution, 50 per cent. w/v, aqueous.

Cupferron solution, 1 per cent. w/v in chloroform.

Hydroxylamine hydrochloride solution, 10 per cent. w/v, aqueous.

Oxine solution, 2 per cent. w/v in 4 per cent. v/v acetic acid.

Oxalic acid solution, 10 per cent. w/v, aqueous.

Ammonium oxalate wash solution—A solution containing 0·1 per cent. of ammonium oxalate in water.

Ammonium dihydrogen phosphate—Analytical-reagent grade.

Ammonium nitrate wash solution—A solution containing 10 per cent. of ammonia solution, sp.gr. 0·880, and 2 per cent. of concentrated nitric acid in water.

DECOMPOSITION OF THE SAMPLE—

Weigh 0·5000 g of sample into a 3-inch platinum basin, add 10 ml of hydrofluoric acid, and set the solution aside overnight. Then add 10 ml of perchloric acid, and evaporate to dryness on a hot-air bath. Add a further 10 ml of perchloric acid and about 20 ml of water, heat on an air bath to dissolve the solids, and evaporate to dryness. Repeat this operation three times to ensure removal of fluoride. Dissolve the residue in 10 ml of 5 N hydrochloric acid, transfer to a 100-ml calibrated flask, and dilute to the mark with water.

PROCEDURE—

Take 50 ml of sample solution, dilute to 100 ml in a 250-ml beaker, add 5 ml of saturated bromine water (to oxidise ferrous iron), boil for 1 to 2 minutes, and then add 1 g of benzoic acid. Add diluted ammonia solution (1 + 1) dropwise, with stirring, until a faint permanent precipitate forms, complete the precipitation by adjusting the pH to 3·5 with ammonium acetate (50 per cent.), boil for about 2 minutes to coagulate the precipitate, and stand the beaker on a boiling-water bath for 30 minutes. Collect the precipitate by filtration through a Whatman No. 540 filter-paper, and wash several times with the ammonium benzoate wash solution. Wash the precipitate back into the beaker, dissolve in 20 ml of hot 5 N hydrochloric acid, dilute to 100 ml with water, and reprecipitate. Set the filtrate aside.

Dissolve the precipitate in 20 ml of 5 N hydrochloric acid, and wash the solution into a separating funnel (total volume should be about 75 ml). Extract iron and titanium with successive portions of a 1 per cent. solution of cupferron in chloroform until the extract is colourless, and wash twice with chloroform and once with light petroleum (it is essential to remove all cupferron, otherwise its oxidation products will contaminate the oxine precipitate). Transfer the aqueous solution to a 250-ml beaker. Add sufficient oxine solution to ensure an excess of 2 mg per ml and 2 ml of hydroxylamine hydrochloride solution (this inhibits the oxidation of reagents and prevents the discoloration of the precipitate); heat the solution to 70° C, and add diluted ammonia solution (1 + 1) until a faint permanent precipitate forms. Precipitate the bulk of the aluminium by slowly adding, with stirring, 10 ml of 50 per cent. ammonium acetate solution, and adjust the pH to about 5 with diluted ammonia solution (1 + 1). Stand the beaker on a water bath for 30 minutes to allow the precipitate to settle. Collect the precipitate in a sintered-glass crucible, wash several times with hot water, dry at 140° C, and weigh. Heat the combined filtrates from the benzoate separations to boiling, and add sufficient 10 per cent. w/v oxalic acid solution to give 1 g of oxalic acid per 100 ml of solution. Adjust the pH of the solution to 4·0 with diluted ammonia solution (1 + 1), boil for 2 to 3 minutes, stand on a water bath for 2 hours, and set aside overnight. Collect the precipitate on a porous porcelain crucible, and wash with a 0·1 per cent. solution of ammonium oxalate. Dissolve the precipitate in hot hydrochloric acid, and wash with water into a 250-ml beaker. Dilute the solution to 100 ml, add 1 g of oxalic acid, heat to boiling, and precipitate the calcium by raising the pH to 4·0 with diluted ammonia solution (1 + 1). Boil the solution for 2 to 3 minutes, stand on a water bath for 30 minutes, and set aside to cool for 2 hours. Collect the precipitate on the porous porcelain crucible, wash with ammonium oxalate solution, ignite at 550° C, and weigh as calcium carbonate.

To remove ammonium salts, concentrate the combined filtrates by evaporation, add 25 ml of concentrated nitric acid and, after the effervescence ceases, evaporate to dryness. Add 100 ml of water, make the solution slightly ammoniacal, and transfer to a 250-ml beaker. Add 1 g of ammonium phosphate and 20 ml of ammonia solution, sp.gr. 0·880, for each 100 ml of solution, and set aside for 2 to 3 days. Collect the precipitate in a porous porcelain crucible, wash with cold ammoniacal ammonium nitrate, dissolve in hydrochloric acid, and reprecipitate. Set the solution aside overnight, and collect the precipitate in the same crucible; ignite the precipitate at 950° C, and weigh as the pyrophosphate. Apply a correction for manganese, which is collected at this point.

Recovery of Aluminium, Calcium and Magnesium by the Proposed Scheme

This systematic scheme was applied to a synthetic solution, and in Table III is shown the recovery of calcium, magnesium and aluminium in the presence of phosphate, titanium and iron.

TABLE III

RECOVERY OF ALUMINIUM, CALCIUM AND MAGNESIUM

The solution contained, beside the relevent elements, 10·0 mg of Fe_2O_3, 4·0 mg of TiO_2 and 75·0 mg of P_2O_5

	Added, mg	Found, mg	Found, mg (corrected)*
Alumina	25·00	24·81	—
Calcium oxide	100·00	99·85	100·18
Magnesium oxide	10·00	10·20	9·98

* Correction applied for calcium found in the magnesium pyrophosphate.

The calcium precipitate, after being weighed as carbonate, was ignited to the oxide and examined spectrographically; no significant amounts of impurities were detected (less than 1 part in 1000). The magnesium pyrophosphate precipitate was also examined spectrographically. Any group-3 elements escaping precipitation as benzoates would be collected with the magnesium precipitate, but none was detected, thus confirming that the precipitation of these elements by benzoic acid was complete; calcium, detected spectrographically, was determined chemically by separating it as the sulphate in 75 per cent. alcoholic solution. The amount found was 0·33 mg, calculated as calcium oxide.

NON-SYSTEMATIC ANALYSIS

The remaining elements are determined on separate portions of solid powder or solution.

Determination of Iron

Hoffman and Lundell[2] determined iron by a standard volumetric method, and Hillebrand and Lundell,[10] in discussing the titanous sulphate method, do not report phosphate as an interfering ion. Colorimetric methods may present difficulties owing to the formation and precipitation of iron^{III} phosphate under the weakly acid conditions often employed. The common method in which the iron^{II} complex of 2,2′-bipyridyl or *o*-phenanthroline is formed, with hydroxylamine hydrochloride being used as a reducing agent in an acetate buffer, is subject to this interference, and Baudemer and Schaible[11] prefer a citrate buffer with hydroquinone as the reducing agent. They emphasise the importance of adding the reducing and colorimetric reagents before the buffer. Grat-Cabanac[12] also reports that, under these conditions, hydroxylamine hydrochloride is not satisfactory, as the colour development takes 20 hours to reach a maximum, and she considers sodium dithionite a better reducing agent.

Our work shows that the order of addition of the reagents and the effectiveness of the reductant are important. To avoid error in this determination iron must be fully reduced to the ferrous state in acid solution (0·2 N hydrochloric acid), before the pH is raised by adding the buffer solution, otherwise recoveries are low; presumably iron^{III} phosphate comes out of solution. We find that in 0·2 N hydrochloric acid solution hydroxylamine is a poor reducing agent at room temperature; hardly any reduction takes place after 10 minutes standing (even after 20 hours some ferric iron remains). These observations show that in the normal method effective reduction must take place simultaneously with the formation of iron^{II} - bipyridyl complex in acetate buffer as colour development is complete in 15 minutes, so that when phosphate is present low results are to be expected. In boiling 0·2 N hydrochloric acid solutions hydroxylamine hydrochloride completes the reduction of iron in 20 minutes and there is no error in the determination.

The use of a more effective reducing agent, ascorbic acid, in place of hydroxylamine hydrochloride also avoids this source of error. A disadvantage is that titanium forms a yellow complex with ascorbic acid, and may cause interference if present in large amount. However, this yellow complex forms only in acetate buffer solution (pH 5·0) and not in chloroacetate buffer solution (pH 3·0). As other group-3 phosphates may precipitate in acetate buffer, the formation of the iron^{II} - bipyridyl complex in chloroacetate buffer is to be preferred.

METHOD

SAMPLE SOLUTION—

Prepare as for the systematic analysis.

REAGENTS—

2,2′-Bipyridyl solution, 0·15 *per cent. w/v, aqueous.*

Chloroacetic acid solution, 1 N.

Ascorbic acid—Analytical-reagent grade.

PROCEDURE—

By pipette, transfer 10 ml of the sample solution, containing not more than 1 mg of Fe_2O_3 to a 50-ml beaker, add 50 mg of ascorbic acid and 10 ml of bipyridyl solution, and set aside for 10 minutes. Add 5 ml of chloroacetic acid solution, and adjust the pH to 3·0 by dropwise addition of 4 N ammonia solution; wash into a 100-ml calibrated flask, and make up to the mark with water. Measure the optical density in a 1-cm cell at 525 mμ. Carry out determinations on a standard solution and a blank solution at the same time.

Determination of Titanium

As the determination of titanium by the peroxide method is often made in phosphoric acid solution to reduce interference by iron, the presence of phosphate in the sample is of little significance. The sensitivity of the method may be reduced by phosphoric acid, but according to Weissler,[13] this is avoided by measuring the optical density at 400 mμ.

METHOD

SAMPLE SOLUTION—

Prepare as for the systematic analysis, but after the final evaporation dissolve the solids in 5 ml of 60 per cent. perchloric acid before diluting to 100 ml in a calibrated flask.

REAGENTS—

Mixed acids—A mixture containing 20 per cent. v/v of 20 N sulphuric acid and 20 per cent. v/v of syrupy phosphoric acid.

Hydrogen peroxide reagent—Mixed acids containing 20 per cent. v/v of hydrogen peroxide (100 volume).

PROCEDURE—

By pipette, place 20 ml of the sample solution into a 25-ml calibrated flask, make up to the mark with hydrogen peroxide reagent, and mix. Repeat, with the mixed acids in place of the hydrogen peroxide reagent to obtain the sample blank solution. Determine the reagent blank value on 20 ml of distilled water. Measure the optical densities of solutions in 4-cm cells at 400 mμ. Deduct both the reagent blank value and the sample blank value, and compare with determinations carried out on standards at the same time.

Determination of Manganese

Determining manganese in phosphate rock raises no special problems, and the permanganate colorimetric method is satisfactory. Various combinations and concentrations of phosphoric, sulphuric and nitric acids have been used, but we find that nitric acid inhibits the oxidation of manganese to permanganate and that this is most marked when the acid contains much nitrogen dioxide; nitric acid was therefore omitted from the acid solutions.

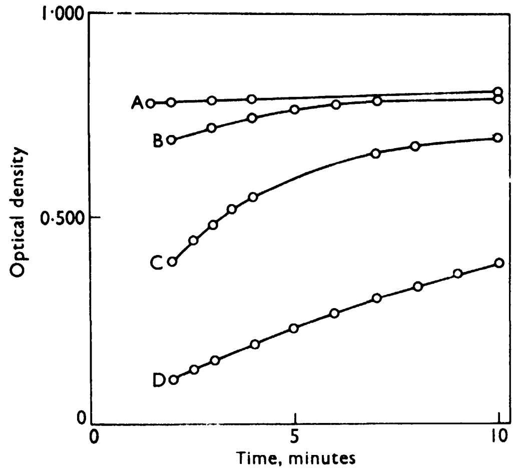

Fig. 1. Effect of fluoride on the rate of formation of silicomolybdic acid. Solutions contained 500 μg of silica, 2 ml of ammonium molybdate solution (10 per cent. w/v in 2 N sulphuric acid) and different amounts of fluoride in a total volume of 25 ml: curve A, no fluoride; curve B, 5 mg of fluoride; curve C, 7·5 mg of fluoride; curve D, 10·0 mg of fluoride.

Optical densities were measured at 400 mμ in 1-cm cells after various intervals of time

METHOD

SAMPLE SOLUTION—

Prepared as for the titanium determination.

REAGENTS—

Mixed acids—A mixture containing 50 per cent. of 20 N sulphuric acid and 25 per cent. of syprupy phosphoric acid.

Sodium periodate—Analytical-reagent grade.

Sodium nitrite solution, 10 per cent. w/v, aqueous.

PROCEDURE—

By pipette, transfer 25 ml of sample solution to a 100-ml beaker, and add 10 ml of mixed acids and 0·3 g of sodium periodate. Boil the solution for 5 minutes after the permanganate colour first appears, allow to cool, transfer to a 50-ml calibrated flask, make up to the mark with distilled water, and set aside overnight. Measure the optical density of the solution in a 2-cm cell at 525 mμ, add one drop of sodium nitrite solution, stir to decolorise, and measure the optical density again. The differences in these optical densities are used in the calculations. Compare with standard solutions.

Determination of Silica

As the gravimetric procedure is difficult and tedious, a colorimetric method based on the formation of the yellow silicomolybdic acid and subsequent reduction to molybdenum blue was adopted. In this method, attention must be paid to the effect of phosphate. Fluoride suppresses the formation of silicomolybdic acid and according to Case[14] this interference can be eliminated by the addition of boric acid. However, Greenfield[15] maintains that no precautions need be taken for phosphate rocks.

Our investigation shows that fluoride interferes by retarding the formation of the silicomolybdic acid (see Fig. 1). This effect will be negligible for the amount of fluoride normally encountered in phosphate rocks, but, for safety, a period considerably in excess of the minimum time required for the formation of the hetero-poly acid was allowed in the procedure described.

Phosphate would cause positive interference, as it forms a hetero-poly acid with molybdate that is reduced to molybdenum blue. Oxalic acid decomposes phosphomolybdic acid and can be used to suppress this interference, but as it also slowly bleaches silicomolybdic acid this procedure must be carefully standardised.

There must be about twice the theoretical amount of molybdate required to form the 12 hetero-poly acids of both silicate and phosphate, otherwise there will be negative interference of the type shown in Fig. 2.

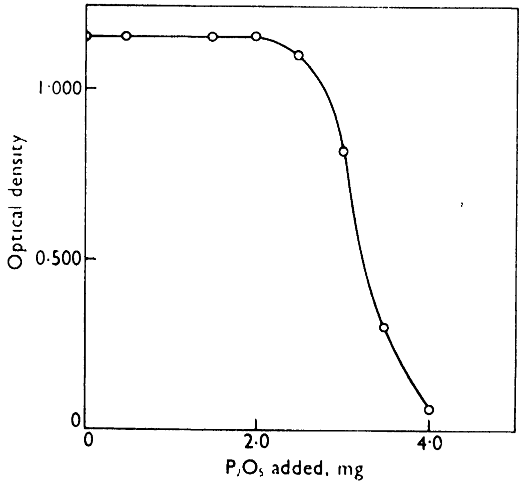

Fig. 2. Interference by phosphate in the determination of silica as molybdenum blue. Silico- and phosphomolybdic acids were formed in solutions containing 300 μg of silica, x mg of P_2O_5 and 1 ml of ammonium molybdate solution (10 per cent. w/v in 2 N sulphuric acid) in a total volume of 25 ml. Reduction to molybdenum blue, after addition of 5 ml of 10 per cent. oxalic acid solution, was by 100 mg of ascorbic acid. The final volume was 100 ml and optical densities were measured at 810 mμ in 1-cm cells

METHOD

REAGENTS—

Sodium hydroxide pellets—Analytical-reagent grade.

Ammonium molybdate solution, 10 *per cent. w/v in* 2 N *sulphuric acid.*

Oxalic acid solution, 10 *per cent., aqueous.*

Ascorbic acid—Analytical-reagent grade.

PROCEDURE—

Weigh 250 mg of sample (—150 mesh) into a silver crucible, add 4 g of sodium hydroxide, and gently heat with constant swirling to a dull red heat; fuse for about 5 minutes. Cool the melt, half fill the crucible with water, digest on a water bath for 1 to 2 hours, and wash into a 1-litre calibrated flask half filled with water containing 10 ml of 12 N hydrochloric acid. Clean the crucible with water containing 2 ml of 12 N hydrochloric acid, add this solution to the contents of the flask, and make up to the mark with distilled water.

Transfer a portion of this solution, containing not more than 300 μg of silica, to a 100-ml calibrated flask, add 1 ml of ammonium molybdate solution (sufficient for about 1·5 mg of phosphate), mix, and set aside for 10 minutes.

Then add 5 ml of oxalic acid solution, mix well, after 30 seconds add 100 mg of ascorbic acid, and dilute to the mark. Allow 2 to 3 hours for the reduction, and measure the optical density in a 1-cm cell at 810 and 650 mμ. Compare with standards after deducting blank values (normally negligible).

Determination of other Elements

Phosphate, carbonate, water and fluoride are determined by the usual methods. Spectrochemical arc techniques are suitable for determining most trace elements, except uranium. The method is not sensitive for this element, which can be determined by separating it from phosphate and most other elements (except iron) by anion exchange and employing a colorimetric finish. The small amounts of aklalis present are best determined by flame-photometric methods. In determining total sulphur, wet oxidation is employed for the decomposition of the sample.

CONCLUSION

A double precipitation of the group-3 elements as benzoates at pH 3·5 effects a good separation of these elements from calcium, magnesium and manganese, in the presence of phosphate. This separation permits a modified standard scheme to be applied to the group analysis of phosphate rocks.

I thank Mr. K. L. H. Murray for the spectrographic analysis of precipitates.

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The Removal of Phosphate in the Barium Perchlorate Titration of Sulphate

Application of the Method to the Oxygen-flask Combustion Technique

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A procedure is described for the quantitative separation of phosphate from mixtures containing sulphuric and phosphoric acids. The free acids are first converted to the corresponding silver salts, and, after separation of the insoluble silver phosphate, the soluble silver sulphate is decomposed by treatment with a suitable ion-exchange resin. The free sulphuric acid produced is then titrated with an aqueous solution of barium perchlorate. In combination with the oxygen-flask combustion technique the method provides an accurate and precise procedure for the micro-determination of sulphur in organic compounds containing phosphorus.

It is now well established that the oxygen-flask combustion technique, in combination with a suitable volumetric procedure, provides the most rapid and convenient method for the micro-determination of halogens, sulphur, phosphorus, boron, etc., in a wide range of organic compounds.[1,2] In determining sulphur for example, the sulphuric acid obtained by decomposition of the sample can be titrated accurately with barium perchlorate,[3] if certain interfering elements, including metals, fluoride and phosphate, are absent. Metals can be removed by a suitable ion-exchange resin,[3] and interference by the fluoride ion can be suppressed by adding boric acid to the solution before titration.[2] The use of magnesium carbonate for removing phosphate has been proposed,[3] but experience suggests that complete separation cannot be achieved with this reagent. From a consideration of the relative solubilities of magnesium and silver phosphates, it was thought that a more complete separation of the phosphate could be effected by precipitation as the silver salt. This was confirmed experimentally by tests carried out with aqueous solutions containing only sulphuric and phosphoric acids. By suitable modification, including the use of a cation-exchange resin, the method was successfully applied to the separation of phosphate, the sulphuric acid then being titrated with barium perchlorate. Finally, in combination with the oxygen-flask combustion technique, the method was successfully applied to the determination of sulphur in organic compounds containing phosphorus.

Experimental

The separation of phosphate from sulphate by precipitation with magnesium carbonate—

The examination of this procedure was limited to the simple experiment described below. An accurately measured volume of 0·025 N sulphuric acid was diluted to 25 ml with water, and, after the addition of 100 ml of ethanol, the solution was titrated with 0·01 N barium perchlorate solution with a mixture of thorin and methylene blue as indicator.[4] The volume of titrant required was 5·38 ml. In a further test, a similar volume of sulphuric acid was mixed with a small volume of phosphoric acid equivalent to 3 mg of phosphorus, and, after dilution to 6 ml, the solution was boiled with 120 mg of magnesium carbonate for a few minutes. After it had been cooled in ice-water for about 10 minutes, the solution was filtered into a flask containing about 20 g of moist Amberlite IR-120 cation-exchange resin, and the filter washed with 20 ml of water. The flask was shaken for 5 minutes, the solution decanted, and the resin rinsed with four 25-ml portions of ethanol. The combined solutions were then titrated as before.

The volume of titrant required was about 6·0 ml and the end-point was poorly defined. In a final experiment this procedure was repeated as far as the decantation of the solution from the resin. The resin was then washed with water instead of alcohol, and the phosphate content of the solution was determined colorimetrically.[5] The amount of phosphorus found was 0·2 mg, and, since this result showed that only about 93 per cent. of the added phosphate had been separated as the magnesium salt, the method was abandoned without further examination.

THE SEPARATION OF PHOSPHATE AS SILVER PHOSPHATE—

Preliminary experiments—Since many anions interfere in the titration of sulphate with barium perchlorate, especially if present in large amount, and since it was desirable that the metal content of the test solutions should be as small as possible at every stage, no attempt was made to use a soluble silver salt for precipitating phosphate from mixtures of sulphuric and phosphoric acids.

It was thought that a satisfactory method of separation might be based on the reaction of the mixture of acids with silver oxide, and this was supported by the result of the preliminary experiment described below. An excess of silver oxide was added to 5·0 ml of a solution containing sulphuric and phosphoric acids equivalent to 0·2 mg of sulphur and 0·6 mg of phosphorus per ml, respectively. The solution was heated with an excess of silver oxide, cooled, and filtered on to a few grams of cation-exchange resin. The mixture was shaken for a few minutes, the solution decanted, and the resin washed repeatedly with water. Colorimetric determination of the phosphorus content of the combined solutions showed that only 0·02 mg, or 0·7 per cent., of the added phosphorus had escaped precipitation as silver phosphate. In further experiments with similar test solutions the same procedure was employed, except that the resin was rinsed with ethanol in place of water and the free sulphuric acid in the combined solution was titrated with barium perchlorate. Unsatisfactory end-points were at first obtained, but it was found that this defect could be overcome by washing the resin with dilute sodium hydroxide solution, water and ethanol, in that order, before use. The relative merits of aqueous and alcoholic solutions of barium perchlorate were also examined at this stage, and no significant difference between them was observed in the titration of free sulphuric acid. The more convenient aqueous solution was therefore used in all subsequent work. With these minor modifications a further series of tests was carried out. For each test, 2·0 ml of a standard solution of sulphuric acid were transferred from a content pipette to a test-tube containing a small volume of phosphoric acid equivalent to 3 mg of phosphorus. The pipette was rinsed with small quantities of water to give a total volume of 10·0 ml, and the solution was heated to near boiling-point. Successive small portions of silver oxide were then added until present in excess, the solution being re-heated to near boiling-point at intervals. The solution was cooled to room temperature, filtered into a flask containing about 20 g of moist Amberlite IR-120 cation-exchange resin, and the filter washed with 15·0 ml of water. The flask was stoppered, shaken for about 5 minutes, the solution decanted, and the resin rinsed with four 25-ml portions of ethanol.

The combined solutions were then titrated with 0·01 N barium perchlorate with a mixture of thorin and methylene blue as indicator. Well defined end-points were obtained in all titrations. The results obtained were—

Sulphur present, mg	0·87
Sulphur found, mg	0·88, 0·87, 0·87, 0·86, 0·88, 0·87, 0·87
Mean value, mg	0·87 Precision (as standard deviation) ± 0·007

APPLICATION OF THE METHOD TO THE OXYGEN-FLASK COMBUSTION TECHNIQUE—

Preliminary experiments—Since hydrogen peroxide was to be used as the absorbent in the oxygen-flask combustion method, the phosphate separation procedure was applied to a series of solutions containing 1·0 ml of 30 per cent. hydrogen peroxide in addition to sulphuric and phosphoric acids equivalent to about 1·0 mg of sulphur and 2·0 mg of phosphorus, respectively. The tests were carried out as described above, except that the solutions were not heated above 40° C until most of the hydrogen peroxide had been decomposed by the silver oxide. This minor modification was necessitated by the violence of the reaction at temperatures close to the boiling-point of the solution. The results obtained were—

Sulphur present, mg	0·875
Sulphur found, mg	0·89, 0·85, 0·90, 0·87, 0·86, 0·86, 0·87, 0·86
Mean value, mg	0·87 Precision (as standard deviation) ± 0·017

In these experiments it was expected that, if the decomposition of hydrogen peroxide proceeds according to the reaction equation—

$$\mathrm{Ag_2O + H_2O_2 \rightarrow 2Ag + H_2O + O_2},$$

the amount of silver oxide required would be much greater than in the absence of hydrogen peroxide, but in fact the amounts required were not widely different. The results of the experiments described below suggest that the decomposition does not proceed entirely in accordance with the above reaction, but is in part effected by the catalytic action of the finely divided silver resulting from it. In the first experiment, silver oxide was added in excess to a solution containing 336 mg of hydrogen peroxide. The insoluble matter was filtered off and washed with water and then with ammonia solution to remove excess of silver oxide. The residual metallic silver was dissolved in nitric acid and the amount of silver present determined gravimetrically as the chloride. The weight of silver found, expressed as mg of silver per mg of hydrogen peroxide, was 0·1 mg, whereas the amount expected, in accordance with the above reaction equation, was 6·35 mg per mg of hydrogen peroxide. The catalytic effect of the finely divided silver was demonstrated by repeating the first experiment up to the isolation of the metallic silver, and then adding a small amount of the finely divided metal to 5 ml of 20-volume hydrogen peroxide. As expected, vigorous decomposition of the peroxide was observed.

METHOD

With the information provided by the investigation of the separation of phosphate as the silver salt, no difficulty was experienced in applying the method to the oxygen-flask combustion technique. The procedure finally adopted is described below.

Sulphur was determined in benzyl disulphide, in the presence of benzene phosphinic acid in an amount equivalent to 2 mg of phosphorus. The results, corrected for blank value of 0·05 ml of 0·1 N barium perchlorate, were—

Sulphur present (calculated) % ..	26·03
Sulphur found, %	26·0, 25·7, 25·8, 25·8, 25·9, 25·8, 26·0, 25·9, 25·7, 25·9, 25·7, 25·9

The mean value was 25·84 and the precision, as a standard deviation, was ±0·11.

In each of these analyses the weights of sulphur and phosphorus present were about 1 mg and 2 mg, respectively. The application of the procedure to the simultaneous determination of phosphorus and sulphur has not been investigated, but this might be achieved by colorimetric determination of the phosphorus in the mixture of silver phosphate and excess of silver oxide, after solution of the precipitate in sulphuric acid.

REAGENTS—

Hydrogen peroxide, 100 *volume*—M.A.R. grade.

Ethanol, absolute.

Barium perchlorate, 0·01 N, *aqueous*—Adjust to about pH 3·0 by adding perchloric acid.

Thorin indicator solution—Dissolve 25 mg of thorin in 5 ml of distilled water.

Methylene blue indicator solution—Dissolve 15 mg of methylene blue in 50 ml of water.

Silver oxide—To a 3·0 per cent. solution of silver nitrate, add a small excess of 1·0 N sodium hydroxide, with continuous stirring. Wash the precipitate repeatedly by decantation with water, and store the product under water.

Cation-exchange resin, Amberlite IR-120(*H*)—Wash about 400 g of the analytical-grade resin with about 700 ml of 3·0 N hydrochloric acid, by the column method, and then wash with 4 or 5 litres of distilled water, and transfer the resin to a 1-litre flask. Remove most of the water by decantation, add about 100 ml of ethanol, and shake thoroughly for a few minutes. Decant the alcohol, and repeat the washing with two further 100-ml portions of ethanol.

Remove the alcohol by repeated washing with water by decantation. Add 400 ml of 0·5 N sodium hydroxide, shake vigorously for about 10 minutes, and decant the slightly turbid solution. Finally, wash the resin with water until all fine suspended particles have been removed, filter on a Buchner funnel, press the drained resin between filter-papers, and store the moist product in a glass stoppered bottle sealed with adhesive tape. One 25-g portion of resin containing about 45 per cent. of water suffices for at least twenty analyses.

PROCEDURE—

Weigh out an amount of sample corresponding to about 1·0 mg of sulphur, and decompose it in an "oxygen flask" containing 5·0 ml of 20-volume hydrogen peroxide as absorbent.

Rinse the stopper and sample holder with not more than 10 ml of distilled water, and boil the solution to reduce its volume to about 10 ml. During this operation, further rinsing of the stopper can be effected by supporting it loosely in the neck of the flask. Cool the solution to about 40° C, and add small portions of an aqueous suspension of silver oxide until effervescence ceases. Heat the solution to near boiling-point, and, with occasional re-heating, add further portions of silver oxide until an excess is present. With a sintered-glass filter, provided with a thin layer of paper pulp, filter the cold solution into a conical flask containing about 25 g of resin previously rinsed with two 20-ml portions of ethanol. Rinse the sample flask with not more than 10 ml of water in successive small portions, and pass the washings through the filter. Repeat this operation with two 5-ml portions of alcohol, and then add about 4·0 ml of water to the filter. Allow the water to remain on the filter for several minutes before draining it off. Stopper the flask, shake it at frequent intervals during about 5 minutes, add 25 ml of ethanol, and decant the clear solution into a second conical flask. Wash the resin by decantation with three 25-ml portions of ethanol, and titrate the combined solutions with 0·01 N barium perchlorate solution, with 0·1 ml of thorin solution and 0·1 ml of methylene blue solution as indicator. Carry out a blank determination in the same manner, omitting only the sample. The blank value should not much exceed 0·05 ml.

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The Determination of Thallium in Urine

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A method has been developed in which Brilliant green is used for the spectrophotometric determination of thallium in urine.

The thallium - Brilliant green complex, formed in the presence of the organic substrate, is extracted into toluene. The optimum conditions for the determination are described, and the sources of various interferences examined.

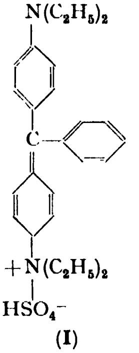
The method has been used to check thallium excretion in the urine of patients.

FOR an evaluation of the effectiveness of chemotherapy in treating thallium poisoning, a rapid method for determining the amount of thallium in the patient's urine is required.

In most existing methods the organic substrate is destroyed as a preliminary step to determining the thallium.[1 to 14] This approach increases the time required for each determination and entails considerably more work. In a few instances the determination of thallium without treatment with mineral acids is described; Stavinoha and Nash[15] extract thallium from urine with an organic solvent and determine it by flame spectrometry, and Reis[16] and Gorzelewska[17] determine extracted thallium spectrophotometrically.

A critical exploration of the approaches available for determining thallium in urine indicated that the method based on the formation of a coloured ion-association complex between a thallic halide anionic complex (TlX_4^-) and the dye Brilliant green (a triphenylmethane derivative) should prove useful.

Brilliant green (I) has colour index number 42040.[18] It is green in aqueous and alcoholic solutions ($\lambda_{max.} = 623$ mμ) and yellow-red in hydrochloric acid solutions. The addition of sodium hydroxide to the aqueous solution results in the precipitation of the bright-green dye base. The reagent was first used by Kul'berg for detecting thallium.[19]


(I)

Reis[16] and Gorzelewska[17] have already used Brilliant green for determining thallium in urine; attempts to repeat their work have, however, been unsuccessful. It was therefore decided to investigate the method systematically, giving due regard to the many variables involved.

Thallium^{III} in halogen acid media forms anionic complexes of the type TlX_4^-,[20] where X indicates the halogen atom. The pertinent formation constants cited in the literature[21] are—

$$\log \beta\ TlBr_4^- = 20{\cdot}4$$

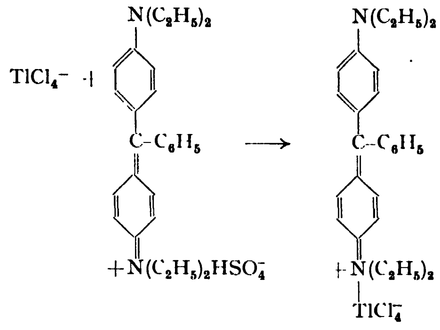
$$\log \beta\ TlCl_4^- = 15{\cdot}4$$

where β is the cumulative constant for the reaction—

$$mM + nL \rightleftharpoons M_mL_n$$

$$\log \beta_{mn} = \frac{[M_mL_n]}{[M]^m[L]^n}$$

An inspection of the above values indicates that the bromide complex will be formed preferentially in media containing high concentrations of bromide. In media containing both chloride and bromide, with the concentration of the former far exceeding the latter, the formation of a chloride or a mixed complex can be expected; both, however, will form ion-association complexes with the dye, as shown below (II) for $TlCl_4^-$.



(II)

This reaction is selective for thallium; the complex formed is coloured and can be extracted with various organic solvents.

EXPERIMENTAL

THE THALLIUM - BRILLIANT GREEN COMPLEX—

A sample of "synthetic urine" to which 10·5 µg of Tl^+ had been added was oxidised with alkaline bromine solution and acidified with hydrochloric acid; Brilliant green dye solution was added and the coloured complex was extracted into toluene. (The "synthetic urine" was prepared by dissolving 38 g of disodium hydrogen orthophosphate, $Na_2HPO_4.12H_2O$, 30 g of sodium chloride and 9·25 g of ammonium chloride in 500 ml of water; 1 ml of this solution contains the average amount of salts present in 5 ml of urine.) The absorption spectrum of the extract is shown in Fig. 1. No change in the optical density of this coloured solution could be detected within 90 minutes of its formation. The optimum amount of reagent was determined by keeping all other variables constant and adding different amounts of reagent; an approximately thousand-fold excess of reagent (1 mg of Brilliant green for each µg of thallium) is necessary to ensure reproducible optical densities. With smaller amounts of the dye, lower optical-density values were obtained.

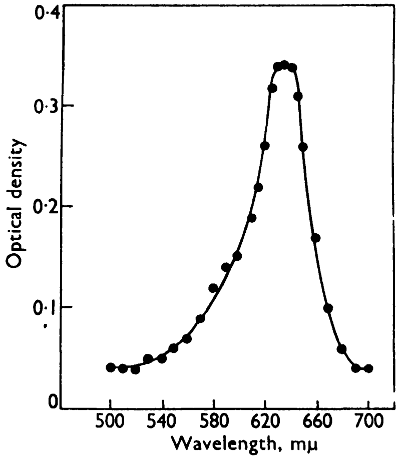


Fig. 1. Absorption spectrum of the thallium chloride - Brilliant green ion-association complex in the toluene extract

OXIDATION OF THALLOUS TO THALLIC—

The oxidation of thallium with sodium nitrite[16,17] did not yield quantitative results (this has also been reported by Campbell[12]); it was therefore decided to investigate the applicability of bromine as oxidant in this method. The presence of halide ions depresses considerably the oxidation potential of the Tl^{3+} - Tl^{+} couple,[22] so that the oxidation of thallous ions by bromine becomes feasible. The presence of excess of bromine must be avoided, since it causes bromination of the reagent, accompanied by the formation of coloured derivatives, some of which are extracted into the organic layer. The presence of appreciable amounts of urea in urine can be exploited for destroying the excess of bromine, especially in an alkaline medium. Twelve millilitres of alkaline bromine solution, prepared as described under "Reagents," were sufficient to effect quantitative oxidation of up to 16 μg of thallous ions present in 15 ml of urine or synthetic urine; for synthetic samples about 0·5 g of urea was added to destroy the excess of bromine, whereas in most samples of urine sufficient urea was present to make this addition unnecessary.

EXTRACTION OF THE COLOURED COMPLEX—

The number of extraction steps recommended was determined on prepared samples composed of 2 ml of synthetic urine, 0·6 g of urea and 8 ml of standard thallium sulphate solution containing 16·2 μg of Tl^{+}. These samples were treated with 5 ml of concentrated hydrochloric acid, 6 ml of alkaline bromine solution and 1 ml of 1 per cent. Brilliant green solution, and extracted with successive 2·5-ml portions of toluene. The organic layers were collected in a centrifuge tube, spun, and their optical densities measured at 640 mμ. Extraction of the ion-association complex formed was quantitative after four successive extractions with 2·5-ml portions of toluene (a further extraction did not appreciably increase the optical densities).

To determine the optimum shaking period, the optical density of the organic layer was measured after various shaking times in the range 30 seconds to 3 minutes. Thirty seconds were found to be sufficient both for samples of synthetic urine and for samples of urine to which thallous ions had been added.

ACIDITY OF THE MEDIUM—

The influence of the acidity of the medium at different stages in the determination was investigated by carrying out a series of blank experiments, with both synthetic urine samples and urine, at various concentrations of hydrochloric acid. Further experiments were made with samples to which known amounts of thallium had been added. It was found that, at acid concentrations up to 1·6 M in the aqueous phase, the dye was extracted into the toluene layer, even in the absence of thallium; at acid concentrations above 3·5 M, on the other hand, the extraction of the coloured thallium - dye complex was less efficient and lower optical densities were obtained. This last effect can be ascribed to competing equilibria, such as the depression of the dissociation of $HTlCl_4$ by the high concentration of hydrochloric acid present.[20,23]

METHOD

REAGENTS—

Standard thallium solution—Dissolve an appropriate amount of thallous sulphate in dilute sulphuric acid and adjust the volume with distilled water. Dilute freshly as required to give solutions containing 4 to 16 p.p.m. of Tl^{+}.

Alkaline bromine solution—Mix 10 ml of saturated bromine water with 2 ml of 25 per cent. sodium hydroxide solution in a small, stoppered Erlenmeyer flask. To avoid introducing bromine droplets with the bromine water, which leads to the addition of an excessive amount of bromine, the saturated bromine water must be prepared, stored and dispensed from the apparatus shown in Fig. 2. Saturation of the bromine water and settling of the bromine droplets is achieved by preparing the bromine water at least 2 days before use. The alkaline bromine solution must be freshly prepared as needed.

Brilliant green solution, 1 per cent.—Dissolve 1 g of the dye in 100 ml of 75 per cent. ethanol, with gentle heating and stirring. This solution is stable for at least 1 month.

Urea solution, 20 per cent., aqueous—Dissolve analytical-reagent grade urea in water, and pass the solution through a column of Amberlite 120-H ion-exchange resin.

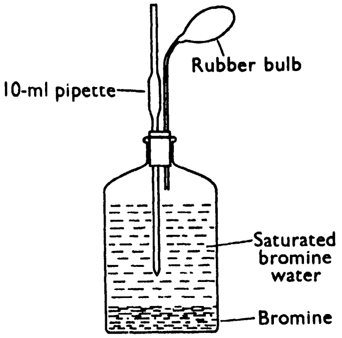


Fig. 2. Apparatus for storing and dispensing bromine water

Toluene—Analytical reagent grade.

Hydrochloric acid, concentrated—Analytical-reagent grade.

APPARATUS—

Spectrophotometer—A Unicam SP500 with 1-cm cells and a Bausch and Lomb Spectronic 20 with 1·17-cm cells were used in the work described here.

PROCEDURE—

Place 10 ml of urine containing not more than 16 μg of thallium (samples containing higher concentrations of thallium must be diluted appropriately) in a separating funnel, add 5 ml of distilled water and then 12 ml of freshly prepared alkaline bromine solution, and cautiously invert the funnel. Add 10 ml of concentrated hydrochloric acid, allow any gases evolved to escape, and again cautiously invert the funnel (it may be necessary at this stage to open the stopper to allow any further gas evolved to escape). Add 2 ml of 1 per cent. Brilliant green solution, and shake the funnel for 30 seconds. (The solution should now be reddish brown; if, however, the reagent has been brominated and the solution has become green, discard the test solution and begin again with a fresh portion of urine to which an appropriate volume of 20 per cent. urea solution—approximately 0·5 g of urea—has been added.)

Extract the coloured complex with four successive 2·5-ml portions of toluene, shaking at each extraction for 2 minutes. Transfer the aqueous layers to a second separating funnel, and collect the toluene layers in a centrifuge tube. When the four toluene layers have been combined, insert the stopper in the tube, and spin in a centrifuge for 3 to 4 minutes at 2000 to 3000 r.p.m. Transfer the clear toluene layer by pipette to a spectrophotometer cell, and measure its optical density at 640 mμ against a toluene blank. Determine the thallium content of the sample with the aid of calibration curves prepared by the same procedure with samples of thallium-free urine to which various amounts of standard thallium solution have been added.

DISCUSSION OF THE METHOD

ADHERENCE TO BEER'S LAW AND CALIBRATION CURVES—

Linear calibration graphs were obtained for samples of synthetic urine and urine to which various amounts of standard thallium solution had been added. These calibration curves, for samples containing up to 16 μg of thallium per 10 ml, agreed with each other within the limits of precision obtainable by this method (see Figs. 3 (*a*) and (*b*)). Each point on these calibration graphs represents the average of a number of parallel determinations; the coefficient of variation of the method was found to be ±10 per cent.

INTERFERENCES—

The influence on the results of some substances, either originally present in the urine samples or introduced in the course of the determination, was investigated. The salt content of urine can differ considerably from sample to sample. Experiments with 10-ml samples prepared by dilution of 1 to 4 ml of the synthetic urine with distilled water showed the presence of a "salting-in" effect, *i.e.*, the optical densities for the higher concentrations exceeded those for the lower concentrations. The loss of precision in the determination of thallium caused by this variation is, fortunately, well within the over-all limits of precision attainable by the method.

The addition, during the determination, of urea to the urine or synthetic urine samples caused excessively high blank optical-density readings, owing to the presence of an unknown contaminant in the analytical-reagent grade urea. Efforts to identify the interfering substance by trace analysis and spot-test methods applied on large samples of urea failed; recrystallisation failed to remove it. Passing the urea solution through a cation-exchange resin was found to provide an adequate method for its purification.

Because of conflicting information[24 to 28] about the selectivity of the method for determining thallium, blank tests were carried out on synthetic urine samples to which various ions had been added. Of all the ions tested (Fe^{2+}, Fe^{3+}, Sn^{2+}, Sn^{4+}, Sb^{3+}, Sb^{5+}, As^{3+}, As^{5+}, Hg^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+}) only antimony, in amounts exceeding 1 p.p.m., and iron, in amounts exceeding 50 p.p.m., interfere.

Since the method is intended for use in clinical laboratories where the cleaning of glassware with detergents is prevalent, the influence of the presence of sulphonated detergents on the determination was tested. Blank determinations carried out with samples containing traces of these detergents resulted in coloured extracts having similar absorption spectra to those obtained in the presence of the thallium - Brilliant green complex. Glassware must therefore be scrupulously rinsed.

Cases of poisoning by heavy metals (and especially lead) have been treated with infusions of solutions of disodium-calcium ethylenediaminetetra-acetate ($CaNa_2$ - EDTA).[29] This treatment is based on the increase in the excretion of lead, owing to the efficient replacement of calcium by lead in the chelate (the formation constant of the lead complex greatly exceeds that of the calcium complex[30,31]). Attempts to treat cases of thallium poisoning by the same method have been made, despite the unfavourable value of the formation constant for thallium.[32,33] To test the efficiency of the proposed procedure in determining thallium in the urine of patients receiving the above treatment, considerable amounts of the disodium ethylenediaminetetra-acetate (1 to 4 ml of a 0·2 M solution) were added to urine samples containing 4 to 16 μg of thallium per 10 ml; these samples were subsequently treated by the proposed procedure, the optical densities were measured and a calibration curve was constructed (see Fig. 3 (*c*)). As could be predicted from the knowledge of the pertinent formation constants and the acidity of the sample solution prescribed in the method, no interference owing to the presence of the chelate could be detected, and the calibration graph obtained corresponds to the normal graph (see Figs. 3 (*a*) and (*b*)).

Clinical applications of the method showed that certain drugs, such as phenacetin, acetylsalicylic acid and antipyrine derivatives, interfered in the determination. Phenacetin and the antipyrines reacted competitively with thallium, thus suppressing the extraction

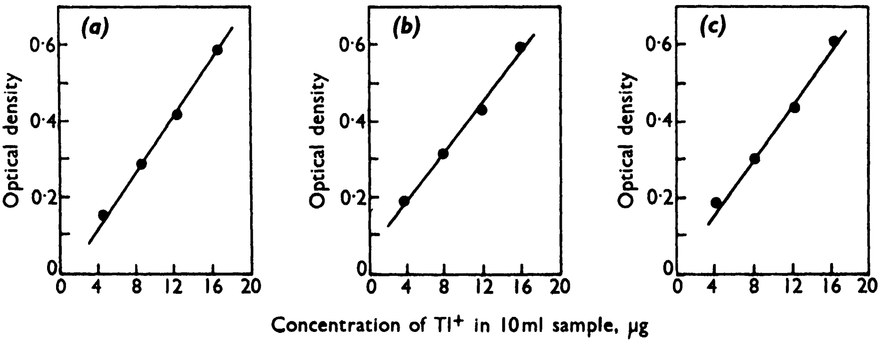


Fig. 3. Calibration curves: (a) 10-ml samples of "synthetic urine" containing 4 to 16 μg of Tl^+; (b) 10-ml samples of urine containg 4 to 16 μg of Tl^+; (c) 10-ml samples of urine containing 4 to 16 μg of Tl^+ to which had been added 4 ml of a 0·2 M solution of disodium ethylenediaminetetra-acetate before the start of the determination of thallium

of the thallium - Brilliant green complex; phenacetin and the acetylsalicylates passed into the organic layer and gave absorption spectra similar to that of the thallium - dye complex. When the patient had been treated with one or more of these drugs, thallium could not be determined in the urine by the direct application of the proposed method; the method could be applied only after removal of the interfering drugs by treating the sample with mineral acids.

CLINICAL TESTS—

The applicability of the method to routine clinical conditions was tested by using it to check the daily excretion of thallium in the urine of three patients. The reliability of the procedure was checked by the method of standard addition. After determining the thallium content of the sample by the direct method and reference to the appropriate calibration graph, a suitable portion of standard thallium stock solution was added to a fresh sample of the same urine, and the thallium content of this mixture was again determined.

The proposed method proved to be sufficiently rapid and sensitive, and its precision was satisfactory for clinical determinations; 0·4 μg of thallium per ml could be determined with a coefficient of variation not exceeding ±10 per cent.

This paper is part of a thesis presented by one of us (D.B.) to the Senate of the Israel Institute of Technology in partial fulfilment of the requirements for the M.Sc. degree.

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Procedure for Routine Determination of Biphenyl and o-Phenylphenol on and in Citrus Fruit*

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An analytical procedure applicable to the routine determination in citrus fruits of the mould inhibitors biphenyl and *o*-phenylphenol is described. Both inhibitors are freed from the bulk of the fruit by steam-distillation before separation of the *o*-phenylphenol by solution in alkali. Biphenyl is determined by its selective ultraviolet absorption after rigorous clean-up; *o*-phenylphenol is determined colorimetrically after coupling with *p*-nitrobenzenediazonium fluoroborate.

THERE is need for an analytical procedure applicable to the routine determination of the widely used mould inhibitors biphenyl and *o*-phenylphenol on and in citrus fruit. Many papers have described analytical procedures for determining one or both of these materials. Colorimetric procedures described for determining biphenyl include the production of a blue colour by reaction with sulphuric acid, formaldehyde and ferric ion[1,2,3] or by reaction with freshly sublimed aluminium chloride[4,5] and production of a purple colour by nitration and then reduction and coupling with *N*-1-naphthylethylenediamine.[6] Instrumental methods for

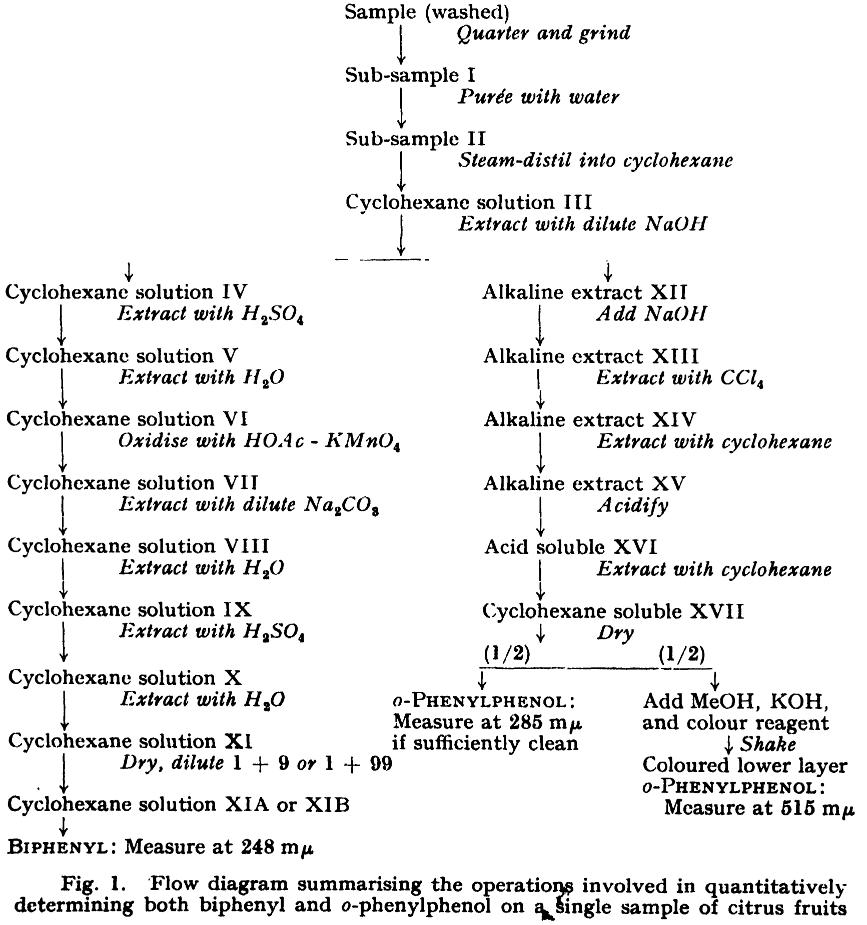

Fig. 1. Flow diagram summarising the operations involved in quantitatively determining both biphenyl and *o*-phenylphenol on a single sample of citrus fruits

determining biphenyl include ultraviolet spectrophotometric measurement at the strong absorption peak at 248 mμ,[7 to 15] infrared spectrophotometric measurement in the absorption band at 14·34 μ[16,17] and gas chromatography with a 6-foot silicone-oil column at 160° C and an argon ionisation detector.[18] The use of "chromatostrips" of activated silicic acid with light petroleum for visual detection of biphenyl in ultraviolet light or for clean-up for ultraviolet spectrophotometric determination[12,13] and paper chromatography of biphenyl after exposure to bromine vapour with ultraviolet detection have also been described.[19] For determining *o*-phenylphenol the colorimetric procedures include the use of 4-aminoantipyrene and potassium ferricyanide[20,21,22,23] coupling with Brentamine fast red GG,[24,25] coupling with diazotised sulphanilic acid[5] and the use of sulphuric acid and titanium sulphate[26] to produce a red colour, the reaction of sulphuric acid and formaldehyde to produce a pink colour[27] and the production of a blue colour by reaction with 2,6-dibromo-*p*-benzoquinonechlorimine.[4,27] Instrumental methods for this compound include ultraviolet spectrophotometric determination in cyclohexane by absorption at 250 mμ[9] and at 284 mμ,[25] in sodium hydroxide solution by absorption at 315 mμ,[25] by the hypsochromic effect from changing pH of its solutions,[25] by measuring the fluorescence of its solution in sodium hydroxide with visual matching of sample with standard solutions[9,25] and by gas chromatography with a 6-foot silicone-oil column at 160° C and an argon detector.[18] Paper chromatography with dilute sodium hydroxide solution to develop the chromatogram and 2,6-dibromo-*p*-benzoquinonechlorimine for detection has also been used.[29]

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* University of California Citrus Research Center and Agricultural Experiment Station Paper No. 1389.

The procedures described here combine selected features of many of the cited procedures and are designed to be adaptable to determining a large number of residues of both biphenyl and *o*-phenylphenol in combination and on a routine basis. In fact, these procedures have been used satisfactorily for this purpose in several regulatory laboratories for some years.[30,31] These procedures utilise ultraviolet spectrophotometry for the final determination of biphenyl and colorimetric coupling of *o*-phenylphenol with the readily available, stable and convenient *p*-nitrobenzenediazonium fluoroborate[32,33] for its determination at 515 mμ. The direct determination of *o*-phenylphenol in citrus extractives by ultraviolet spectrophotometry was unsatisfactory owing to the highly variable background contributions from this substrate. A flow diagram summarising the operations involved is shown in Fig. 1.

METHOD

APPARATUS—

All glassware and other equipment must be scrupulously clean. The recommended procedure is to flush thoroughly and successively with water, 95 per cent. ethanol or acetone and then water; the glassware is then rinsed with 95 per cent. ethanol and then water. Occasionally, charring will occur in the boiler flasks; if this happens, flush out all loose material and add about 20 g of crystalline trisodium phosphate and about 100 ml of water, and set aside overnight before cleaning in the usual manner. All stopcocks must be lightly lubricated with a high-vacuum-type silicone grease, including those in separating funnels.

Meat grinder, with 5-mm holes in grinding plate.
Blender, Waring-type, with 1000-ml jar.
Clevenger trap, modified (see Fig. 2).
Heater, giant cone with rheostat control, or equivalent high-capacity heater.
Shaking machine.
Pipette, 5·0 ml, with lower 1 cm of tip bent at 30° angle.
Spectrophotometer, suitable for measuring optical densities at 248 and 540 mμ.

REAGENTS—

All reagents must be of analytical grade unless otherwise specified.
Sulphuric acid, concentrated.
Antifoam agent—Dow-Corning Antifoam A was used in this work.
Cyclohexane—Spectrophotometric grade.
Sodium hydroxide solution, 0·5 per cent. w/v.
Potassium permanganate solution, 6 per cent. w/v.
Acetic acid, glacial—Redistil at atmospheric pressure in all-glass apparatus before use.
Sodium carbonate solution, 5 per cent. w/v.
Sodium sulphate, anhydrous, granular.

Sodium hydroxide pellets.

Carbon tetrachloride—Redistilled at atmospheric pressure.

Potassium hydroxide solution, methanolic—Dissolve 0·34 g of potassium hydroxide in 1000 ml of methanol.

Colour reagent—Dissolve 0·10 g of *p*-nitrobenzenediazonium fluoroborate in 100 ml of methanol immediately before use. Use freshly prepared solution only.

n-Hexane—Redistil at atmospheric pressure.

Hydrochloric acid, 6 N.

PROCEDURE—

Processing and steam-distillation—Select fruits at random. For the minimum size of sample use 15 grapefruits, 40 lemons, 30 oranges or 50 tangerines.

Rinse the fruits in tap-water, drain them briefly, cut them into polar quarters, and grind alternate quarters in the meat grinder. Mix the ground material thoroughly, and sub-sample by quartering to two sub-samples each of 450 ± 1 g. Fill a 1000-ml graduated cylinder with water, and add about 200 ml of this water to the clean jar of the blender. Transfer one of the sub-samples quantitatively to the jar, stir the mixture for a few seconds at low speed, add more water from the graduated cylinder until the jar is about $\frac{3}{4}$ full, and then blend at high speed for 10 ± 1 minute. If the mixture in the jar is too concentrated, the final purée will become hot enough to evolve steam and thus lose some biphenyl and *o*-phenylphenol; if this occurs, divide future sub-samples between two blender jars, with consequently greater dilution in each.

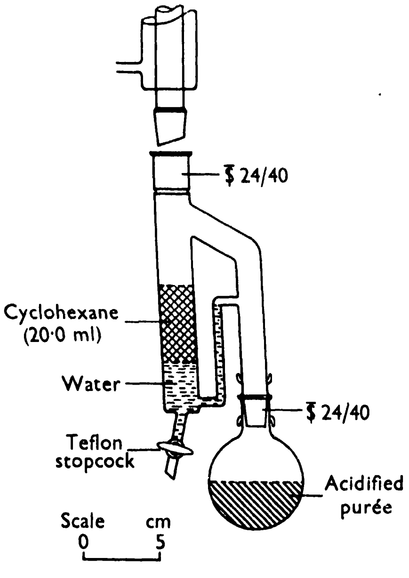

Fig. 2. Modified Clevenger trap

Transfer the resulting purée quantitatively to a clean tared 2-litre graduated cylinder with the remainder of the original litre of water, and weigh to the nearest 3 g to obtain the weight of diluted purée; volume measurements are invalidated by the presence of foam. To a clean round-bottomed 2-litre flask add 3 or 4 carborundum chips, 2 ± 0·2 ml of concentrated sulphuric acid and 0·5 ± 0·2 g of antifoam agent; weigh the flask to the nearest gram. Add to this flask $\frac{1}{3}$ by weight, to the nearest gram, of the thoroughly mixed diluted purée.

In the meantime, arrange the operating condenser, modified Clevenger trap, flask and heater on a ring stand so that the heater and flask can be removed without disturbing the condenser and trap.

Fill the clean trap with distilled water to the overflow point, and attach the flask containing the sub-sample. Carefully transfer by pipette 20·0 ml of spectrophotometrically pure cyclohexane through the upper trap opening on to the surface of the water in the trap. Move the pre-heated heater into position with a piece of wire gauze between it and the flask, and boil under reflux vigorously for 2 ± ¼ hour from the time boiling begins, care being taken to control excessive foaming. At the end of this period, remove the heater, and drain the contents of the trap from the drain cock into a 100- or 125-ml separating funnel. Rinse the trap through its upper opening with 5·0 ml of the pure cyclohexane by means of the special pipette, and put these rinsings in the separating funnel; discard the aqueous layer.

Each millilitre of this final cyclohexane solution now represents 6·00 ± 0·05 g of the parent sample of ground fruit. This solution may be stored awaiting further treatment.

Separation of biphenyl from o-*phenylphenol*—The cyclohexane solution contains biphenyl, *o*-phenylphenol, citrus oils, waxes, other citrus extractives and traces of emulsified water. The desired phenol is separated from this mixture by extraction into dilute alkali. Extract the cyclohexane solution from the steam-distillation with five approximately 10-ml portions of 0·5 per cent. sodium hydroxide solution. There will be considerable emulsification during the first and second extractions only; do not withdraw the emulsion with the aqueous layer. Combine the alkaline extracts from a given sample, and store them in a tightly capped bottle in a refrigerator to await the determination of *o*-phenylphenol.

Ultraviolet spectrophotometric determination of biphenyl—Repeatedly extract the phenol-free cyclohexane solution with approximately equal-volume portions of concentrated sulphuric acid until a fresh acid layer remains clear and colourless after having been shaken; from 5 to 8 extractions will be required. Wash the cyclohexane solution three times with approximately equal-volume portions of water, and transfer it through the neck of the separating funnel to a glass-stoppered 500-ml Erlenmeyer flask; do not rinse the funnel with further cyclohexane, as this would invalidate the weight - volume relationships. To the contents of this flask add approximately 25 ml of the potassium permanganate solution and approximately 25 ml of the glacial acetic acid. Insert the stopper in the flask, holding it tightly in place with a rubber band if necessary, and shake the flask vigorously for 30 + 5 minutes.

Transfer this oxidation mixture to a clean separating funnel, and drain and discard the aqueous layer. Wash the remaining cyclohexane layer twice with approximately 10-ml portions of the sodium carbonate solution, and then once with approximately 25 ml of water. Repeatedly extract this washed cyclohexane solution with approximately 20-ml portions of concentrated sulphuric acid until a fresh acid layer remains clear and colourless after having been shaken; from 3 to 6 extractions will be required.

Wash the cyclohexane layer three times with approximately equal-volume portions of water, and decant it into a storage bottle containing approximately 3 g of the anhydrous sodium sulphate.

After at least 10 minutes, withdraw 1·0 ml of this solution, transfer to a 10-ml calibrated flask, and make up to the mark with the spectrophotometrically pure cyclohexane.

Rinse a silica spectrophotometer cell at least four times with about 1-ml portions of this diluted solution. Fill the cell, and measure the optical density or transmission at 248 mμ, with the pure cyclohexane in the reference cell. If the optical density is less than 0·046 (transmission more than 90 per cent.), use the undiluted solution; if the optical density is more than 0·743 (transmission less than 18 per cent.), use the diluted solution further diluted (1 + 9).

Determine the concentration of biphenyl (in p.p.m.) from a standard calibration graph for purified biphenyl in the pure cyclohexane, with correction for the final dilution. The original 25·0 ml of cyclohexane represents 150 g of ground fruit, or 6 g per ml. Dilution (1 + 9) gives 0·6 g per ml and further dilution of (1 + 9) gives 0·06 g per ml.

Colorimetric determination of o-*phenylphenol*—Add approximately 2 g of the sodium hydroxide pellets to the storage bottle containing the combined 0·5 per cent. alkaline extracts of the original cyclohexane trap solution from the steam-distillation. After the pellets have dissolved, transfer the solution quantitatively with the aid of a few millilitres of water to a clean separating funnel. Extract this solution thoroughly four times with approximately 10-ml portions of the carbon tetrachloride, discarding the lower layer. Extract the remaining aqueous solution once with approximately 10 ml of n-hexane; transfer the lower aqueous layer to a clean separating funnel, and discard the hexane solution. Carefully and slowly add 25 ml of the 6 N hydrochloric acid solution to the alkaline solution in the separating funnel, and then extract the resulting acidified solution with 20·0 ml of n-hexane; discard the lower aqueous solution.

Transfer 10·0 ml of the hexane solution to a clean dry separating funnel. Add 3·0 ml of the methanolic potassium hydroxide solution and 1·0 ml of the freshly prepared colour reagent; shake the resulting mixture thoroughly, and set it aside for about 10 minutes for the colour to develop. Transfer the clear red lower layer to a cell, cap the cell, and measure the optical density or transmission at 540 mμ, with the yellow reagent blank solution (prepared by the same procedure from 10·0 ml of n-hexane) in the reference cell. If the red lower layer is cloudy owing to emulsification, gentle warming of the solution will provide optical clarity. If the optical density is more than 0·743 (transmission less than 18 per cent.), use a smaller volume of solution to achieve an optical density reading of between 0·046 (90 per cent. transmission) and 0·743 (18 per cent. transmission). Dilution with a mixture of methanolic potassium hydroxide and colour reagent causes deviation from Beer's law.

Determine the concentration of *o*-phenylphenol (in p.p.m.) from a standard calibration graph prepared daily from a graded series of dilutions of *o*-phenylphenol in pure cyclohexane, treated as described above with the methanolic alkali and the colour reagent; this curve should cover the range 0·0 to about 10 p.p.m.

DISCUSSION OF THE METHOD

BIPHENYL—

The ultraviolet absorption spectra for several concentrations of biphenyl in spectrophotometric-grade cyclohexane are shown in Fig. 3. Measurement was made at the strong absorption peak at 248 mμ. A calibration curve prepared from Fig. 3 for biphenyl in cyclohexane has a slope of 0·9 μg of biphenyl per ml of cyclohexane solution per 0·1 unit of optical density. Since 1 ml of cyclohexane solution represents 0·06 g of fruit after dilution 1 + 99, this represents 15·6 p.p.m. of biphenyl per 0·1 unit of optical density. This is adequately sensitive for regulatory work as can be seen by the several tolerances for biphenyl in citrus fruits shown in Table I.

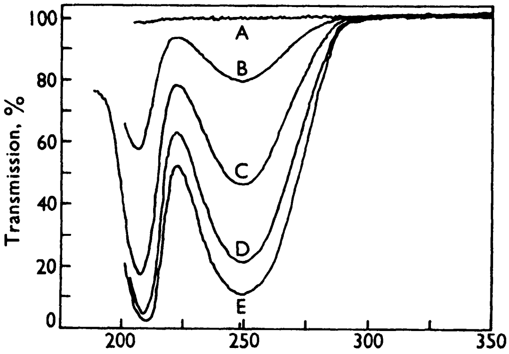

Fig. 3. Standard spectra for biphenyl in cyclohexane: curve A, no biphenyl; curve B, 0·9 μg per ml; curve C, 3·2 μg per ml; curve D, 6·3 μg per ml; curve E, 9·0 μg per ml

TABLE I

TOLERANCES FOR BIPHENYL AND *o*-PHENYLPHENOL IN CITRUS FRUITS

Country	Biphenyl, p.p.m.	*o*-Phenylphenol, p.p.m.
Canada	110	10
England	110*	
France	70	—
Germany	70	10
U.S.A.	110	10

* Total for biphenyl *plus* *o*-phenylphenol.

In Table II, the background values are shown for apparent biphenyl found in untreated fruit and recoveries achieved from fortified fruit. As is evident from the results, recoveries are essentially quantitative. The ultraviolet spectrum for untreated citrus fruit is shown

TABLE II

BACKGROUND VALUES AND RECOVERIES FOR THE BIPHENYL PROCEDURE

Fruit	Background, p.p.m.	Biphenyl added, p.p.m.	Recovery, %
Lemons	1·82 ± 0·38	150	100 ± 1
		41	99 ± 1
Navels	0·95 ± 0·20	150	99 ± 1
		55	100 ± 1
Valencias	3·14 ± 0·88	150	100 ± 1
		50	98 ± 1

in Fig. 4. While the magnitude of this background was negligible for regulatory purposes, the nature of ultraviolet absorbing material was investigated. By means of gas chromatographic isolations and subsequent comparative infrared, ultraviolet, and nuclear magnetic resonance spectrometry it was found that this background absorption was due to *p*-cymene.

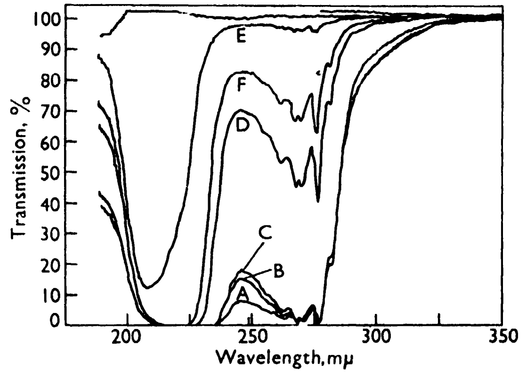

Fig. 4. Biphenyl background spectra for untreated lemons: curve A, sample 26; curve B, sample 27; curve C, sample 28; curve D, sample 28 diluted (1 + 4); curve E, sample 28 diluted (1 + 99); curve F, sample 28 diluted (1 + 9)

o-PHENYLPHENOL—

In Fig. 5 are shown the absorption spectra of the colour produced from several concentrations of *o*-phenylphenol by the colorimetric procedure described. Measurement is made at the strong absorption peak at 540 mμ. A calibration curve for *o*-phenyphenol,

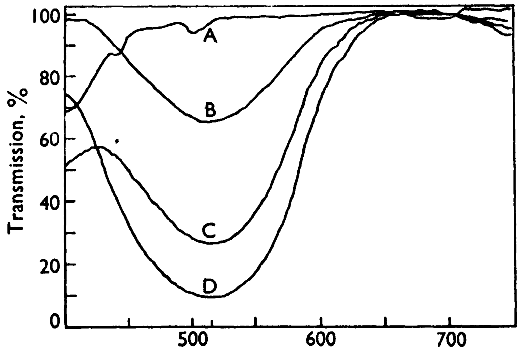

Fig. 5. Standard spectra for *o*-phenylphenol carried through the colour-developing reaction without dilution: curve A, no *o*-phenylphenol; curve B, 8·4 μg per 4 ml; curve C, 21·0 μg per 4 ml; curve D, 42·0 μg per 4 ml

prepared from Fig. 5, has a slope of 4·52 μg per 0·1 unit of optical density; or for a 150-g sample, 0·06 p.p.m. per 0·1 unit of optical density. The colour produced is adequately stable for from 5 to 40 minutes after colour development (apparent concentrations = +1·5 per cent. after 40 minutes).

o-Phenylphenol cannot be steam-distilled so readily by the proposed method and is more soluble in water than is biphenyl. The cyclohexane trapping solution will contain all the biphenyl present, but, when the citrus purée is steam-distilled under the recommended conditions, this trap solution will contain from 80 to 100 per cent. of any *o*-phenylphenol present, depending upon several factors difficult to control. More consistent yields are obtained by carrying out the distillation as directed, and then replacing the cyclohexane trapping solution by fresh cyclohexane for further steam-distillation with subsequent combination of the two distillates. Alternatively, the distillable citrus oils have been used as the sole trapping solvent with excellent recoveries of *o*-phenylphenol.[29] In Table III are shown the background values of apparent *o*-phenylphenol found in untreated fruit by the proposed procedure. Recoveries achieved ranged from 89 to 100 per cent. with from 0·5 to 10 p.p.m. of *o*-phenylphenol.

TABLE III

BACKGROUNDS FOR *o*-PHENYLPHENOL PROCEDURE

Fruit	Background, p.p.m.
Lemons	0·04 ± 0·01
Navels	0·03 ± 0·01
Valencias	0·04 ± 0·01

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A Rapid Assay of Radioactive Caesium in Milk

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A rapid method for the assay of radioactive caesium in milk is described. This involves ashing the sample and separating caesium by allowing a solution of the ash in nitric acid to flow through a bed of ammonium 12-molybdophosphate. The assay is completed by beta-counting the bed of ammonium 12-molybdophosphate.

THE routine assay of radioactive nuclides in milk and other foodstuffs is a long and tedious process. Radioactive strontium is determined by a method based on precipitation (together with an isotopic carrier) by nitric acid, radiochemical purification, the establishment of secular equilibrium between strontium-90 and its daughter element yttrium-90, the separation of parent and daughter elements, and counting. Caesium-137, which is not considered to be so hazardous a nuclide as strontium-90, is determined either on the residues after the removal of strontium nitrate or by direct gamma-ray spectrometry on the original sample; however, this latter method requires expensive equipment and may yield poor counting statistics.

The cation-exchange properties of ammonium 12-molybdophosphate have lately received a great deal of attention from several authors.[1,2,3,4] A method has recently been proposed for the assay of caesium-137 in rain water by allowing the acidified sample to flow through a pre-formed precipitate of the compound supported in a small de-mountable Buchner funnel, and then beta-counting the bed of ammonium 12-molybdophosphate.[5] Since the caesium is so selectively removed from acid solution in this way, it appears feasible that the normal radiochemical assay procedure for milk can be reversed and the caesium-137 determined, before the separation of radioactive strontium, by means of a bed of ammonium 12-molybdophosphate. The method described below enables radioactive caesium to be assayed in 3 days or even less; radioactive strontium can be determined in the usual way after radioactive caesium has been removed from solution.

In an emergency, when many samples contaminated by fission products need to be processed quickly, a rapid assay of caesium-137 may prove useful as a preliminary "screen"; under such conditions, the much larger activities involved would permit a considerable reduction in sample size, more rapid ashing, and a still more rapid estimation to be made. Further, the information obtained may prove a more reliable guide to general fission product contamination than a gross beta-count (when possible) of a sample of ash.

PROCEDURE—

Liquid milk will not readily pass through a bed of ammonium 12-molybdophosphate and, in any case, curdles when acidified with dilute nitric acid. It is therefore necessary to ash the sample. The method adopted for this is based on that of Murthy and Campbell[6]; milk is allowed to flow under gravity from a polythene reservoir, through a polythene tube cooled in ice-water (to prevent coagulation of the milk owing to heat) and to drip into a rotating silica dish heated by two Meker burners. By suitable adjustment of the flow, the rate of rotation of the dish (about 10 to 15 r.p.m. is suitable) and the burners, the milk is immediately evaporated and partly ashed. The temperature must be maintained below 450° C in order to avoid loss of caesium. Ashing is then completed in a muffle furnace at 400° to 450° C in about 48 hours.

Samples of dried milk are charred at 100° to 150° C, and ashing is completed at 400° to 450° C as for liquid milk.

The ash is then damped with a solution containing 1 mg of caesium cations and carriers for any other nuclides to be assayed, and sufficient concentrated nitric acid is added to dissolve the ash. Finely divided carbon is invariably present. Most of this is removed by filtering the solution through paper pulp; no significant retention of caesium-137 by this carbon has been detected.

After concentration of the filtered solution to about 60 ml and adjustment of the nitric acid concentration to between 2 and 8 M, caesium is removed by passage through a bed of ammonium 12-molybdophosphate prepared as described by Broadbank, Dhabanandana and Harding[5]; the filtrate may be used to determine radioactive strontium, or other radionuclides. The bed is sucked almost dry, the funnel carefully de-mounted and caesium-137 determined by counting the filter-paper and the bed of ammonium 12-molybdophosphate under an end-window counter.

COUNTING—

Beta-counting via a thin end-window Geiger counter is convenient and efficient. Unfortunately, however, recovery of caesium-137 from labelled milk samples is not complete. On the other hand, counting with a gamma-scintillation counter (under the photopeak, to reduce background) indicates quantitative recovery of caesium-137. This discrepancy is thought to be due to the presence of an extremely thin film of finely divided carbon on the surface of the bed of ammonium 12-molybdophosphate; the maximum beta energy of caesium-137 - barium-137*m* is comparatively low and even a small thickness of absorber is significant. Removal of carbon, following dissolution of the milk ash, by filtration through paper pulp is more rapid and complete than by filtration under reduced pressure through a filter-paper. This leads to higher apparent recoveries of caesium-137, as determined by beta-counting (see Table I). This apparent recovery is also dependent on the amount of sample taken. Presumably, the distribution of carbon particle sizes in the ash obtained under given conditions is approximately constant and, therefore, the thickness of the carbon film on the bed of ammonium 12-molybdophosphate is approximately proportional to the weight of ash taken (compare Tables I and II).

A further disadvantage of beta-counting is that careless handling of the bed of ammonium 12-molybdophosphate will cause it to break up; this introduces a variable amount of self-absorption by the source, since the caesium is originally present in the surface layers of the bed (see, for example, sample 3 in Table I).

TABLE I

THE RECOVERY OF CAESIUM-137 FROM LABELLED MILK ASH

			Recovery of caesium-137 by—	
Sample number	Beta counts per minute	Gamma counts per minute	beta-counting, %	gamma-counting, %
Reference	1365 ± 11·8	397·3 ± 6·5	—	—
1	1163 ± 10·9	391·9 ± 6·4	85	99
2	1192 ± 11·0	406·9 ± 6·5	87	102
3	975 ± 10·0	393·2 ± 6·4	71*	99
4†	1113 ± 10·7	400·2 ± 6·5	81	101
5†	1047 ± 10·2	381·3 ± 6·2	77	96

* Ammonium 12-molybdophosphate bed broken up.
† Carbon removed by filtration through Whatman No. 1 filter-paper instead of paper pulp.

TABLE II

THE RECOVERY OF CAESIUM-137 FROM LABELLED DRIED MILK

			Recovery of caesium-137 by—	
Sample number	Beta counts per minute	Gamma counts per minute	beta-counting, %	gamma-counting, %
Reference	1455 ± 9·4	616 ± 9·0	—	—
1*	1006 ± 7·8	—	69	—
2*	988 ± 7·7	606 ± 9·0	67	98
3†	952 ± 7·6	560 ± 8·6	65	91
4†	1069 ± 8·0	588 ± 8·8	73	95

* 1 mg of caesium carrier added before ashing.
† 1 mg of caesium carrier added after ashing.

However, in spite of these disadvantages, beta-counting is generally preferred. The adoption of a standardised technique and sample weight yields consistent recoveries of caesium-137, and the high counting efficiency coupled with the low background (especially with a guard tube in anti-coincidence) of the Geiger counting assembly are particularly useful where low activities are to be measured. In an emergency, when relatively high activities are involved, the less efficient gamma-counting may be preferable.

RESULTS

LABELLED MILK ASH—

The results in Table I, for 10 g of labelled milk ash, were obtained by the procedure described below.

Beta-counting was carried out under a Mullard end-window Geiger counter, type MX123, with the sample covered with cellulose tape and on the second shelf of a square lead castle (ERD pattern). The counting efficiency of this arrangement, determined by counting a bed of ammonium 12-molybdophosphate through which a known amount of caesium-137 had been passed under conditions of complete retention, was approximately 6 per cent. Transferring the sample to the top shelf of the castle increased this figure to about 17 per cent., and omission of the cellulose tape increased it further to about 24 per cent.

Gamma-counting was carried out with the same sample on the top shelf of the same castle, under a sodium iodide crystal (1 inch × 1½ inch diameter) in conjunction with an Isotope Developments Limited scintillation head, type 653; a single-channel pulse height analyser was used to allow counting to be carried out under the 0·66-MeV photopeak of caesium-137 - barium-137*m*. The counting efficiency was determined, as described in the previous paragraph, to be about 2 per cent.

Completeness of recovery was judged by comparing count-rates of samples (each of which contained about 10^{-5} mC of caesium-137) with that of a bed of ammonium 12-molybdophosphate through which the same amount of caesium-137 had been passed under conditions of complete retention.

TABLE III

CAESIUM-137 IN UN-LABELLED DRIED MILK

Sample	Caesium-137 content found by— proposed method, pC per kg	direct gamma-ray spectrometry,* pC per kg
A	574 ± 15	585
B	1073 ± 15	943
C	657 ± 28	778

* We are grateful to the Agricultural Research Council Radiobiological Laboratory for these samples. Comparable errors are not quoted for the gamma-ray spectrometric measurements.

All counting results have been corrected for background (about 14 c.p.m. for the Gieger counter and 10 c.p.m. for the scintillation counter).

LABELLED DRIED MILK—

The results in Table II, for 500 g of labelled dried milk, were obtained by the procedure described below.

One millilitre of caesium-137, with or without caesium carrier, was thoroughly mixed with a cream prepared from 500 g of dried milk and distilled water. After the cream had been dried, it was ashed at 400° to 450° C for 48 hours, caesium carrier was added, if this had been omitted initially, and the 25 to 30 g of ash were treated as described above for labelled milk ash.

The loss of caesium, as determined by gamma-counting, is small, but the lower apparent recovery of caesium by beta-counting, as compared with the smaller ash samples of Table I, is apparent. Whether the caesium carrier is added before or after ashing appears to have little influence on the results.

UN-LABELLED DRIED MILK—

The results in Table III were obtained with 500-g samples of dried milk, whose caesium-137 content had already been determined by direct gamma-ray spectrometry (we are indebted to The Agricultural Research Council Radiobiological Laboratory, through Dr. J. D. Burton, for these samples). In all tests, the final beds of ammonium 12-molybdophosphate were counted, uncovered, under a Mullard Geiger counter, type MX123, on the top shelf of a square lead castle (ERD pattern). Sample *plus* background and background alone were counted alternately for 10^3 seconds until at least ten readings for each had been obtained. The standard deviations quoted in Table III are based on the standard deviations of the difference between the means of the two sets of count-rates.

For evaluation of activities from count-rates, a known volume of a reference solution of caesium-137 was used and the percentage recovery was taken as 65 per cent., as seemed reasonable from a consideration of Table II.

INTERFERENCE FROM POTASSIUM

The isotope potassium-40, which occurs to the extent of 0·0118 per cent. in natural potassium, is radioactive. Since ordinary milk contains about 1·4 g of potassium per litre, its radioactivity due to potassium-40 is far greater than that normally due to caesium-137. Owing to the similarity in the chemical behaviour of the two elements, it is of interest to examine the possibility of interference by potassium in the assay of radioactive caesium.

A solution of 2 g of potassium nitrate in 2 to 8 M nitric acid was passed through a bed of ammonium 12-molybdophosphate, which was then sucked dry and counted under an end-window Geiger counter, the background of which had been reduced to 1·4 c.p.m. by an anti-coincidence arrangement. A count rate of less than 0·5 c.p.m. above background was recorded over a counting period of 4½ hours.

If the whole of the ammonium ions in the ammonium 12-molybdophosphate were replaced by potassium, then the molybdophosphate bed would contain a little more than 7 mg of potassium and the potassium-40 content would give about 12 disintegrations per minute. This corresponds, at a counting efficiency of 20 per cent., to about 2·5 c.p.m., which represents an amount of caesium-137 smaller than the standard deviations reported in Table III.

CONCLUSIONS

The procedure described allows the assay of caesium-137 in milk and dried milk to be carried out rapidly and with simple and inexpensive counting equipment. The accuracy and precision of the results are adequate for most purposes.

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We also thank Mr. E. R. Mercer and Dr. J. D. Burton of the Agricultural Research Council Radiobiological Laboratory for their co-operation.

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A Liquid-scintillation Method for determining Tritium in the Tissues of Animals dosed with Tritium-labelled Vitamin A

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A liquid-scintillation counting method is described for determining tritium as tritium-labelled vitamin A in suspensions of tissue, blood and faeces.

Some problems of the method are examined, *e.g.*, the influence on the measurements of the composition of the scintillator and the auxiliary compound, anhydrous sodium sulphate. The use of an internal standard is described, and the range of error and the evaluation of the results are discussed.

Finally, the procedure is described in detail and results are reported that show good agreement between this method and the conventional method for determining vitamin A.

THIS paper describes the development of an extremely sensitive method for determining tritium as vitamin A and its decomposition products in tissue and faeces. These investigations are a continuation of earlier absorption experiments with vitamin A.[1,2]

Vitamin A may be labelled with carbon-14 or tritium, the radioactive isotope of hydrogen. The labelling of organic compounds containing hydrogen can be carried out fairly easily[3]; further, in biological experiments it is possible to use this isotope in high activities, because of its weak irradiation. For this reason tritium-labelled vitamin A was used in these experiments. Previously, the determination of tritium was considered difficult because of its weak irradiation. To-day these difficulties have been overcome, as tritium can be determined by means of liquid-scintillation counting. By this method the radioisotope is added to a solution containing an organic compound, "the liquid scintillator," which will, under the influence of the ionising radiation from tritium, emit light photons. The light is absorbed by a photocell (photomultiplier). Each light quantum gives rise to an electric pulse, which, after amplification, is counted.

In the liquid-scintillation method it is not always necessary to isolate the tritium-labelled compound, as it is possible to measure the tritium activity directly in the tissues. Tissue can be dissolved in formamide by heating. A turbid solution is thus formed and measurements are made after dispersing it in a solution of liquid scintillator.[4,5,6,7] Passmann, Radin and Cooper[8] introduced the high molecular-weight quaternary ammonium base, hyamine, as a solvent for carbon dioxide; this compound has since been used successfully as a solvent for tissue. Agranoff[9] briefly states that freeze-dried tissue powdered and dissolved in hyamine exhibits a slight colour. Herberg,[10] however, mentions that coloured materials must be removed with hydrogen peroxide and that phosphorescence must be overcome by acidifying with concentrated hydrochloric acid, which may cause some precipitation. The preparation procedures are often long and time-consuming.

EXPERIMENTAL

The soft β-radiation from tritium has been measured in tissue and faeces as described below. The minced tissue is ground with anhydrous sodium sulphate, and the resulting powder is suspended in a thixotropic gel containing the scintillator. The suspension is measured in a liquid-scintillation counter, with attention to quenching, light-absorption and phosphorescence.

SOME PROBLEMS OF THE METHOD—

The liquid scintillator—The liquid scintillator fluid should be able to count all the different fractions, *i.e.*, aqueous solutions, as well as organic fluids and tissue particles; a gel can be used for the tissue particles. White and Helf[11] have described a thixotropic gel formed by adding Thixcin (Baker Castor Oil Company) to an organic fluid. Helf[12] states that at a concentration of 25 g of Thixcin per litre of fluid, 22 ml of gel will keep 1 g of material suspended. Helf further mentions that the addition of Thixcin to ordinary scintillators decreases the optical transmission by up to 10 per cent. when carbon-14 is being measured. Furst, Kallmann and Brown[13] mention that the counting efficiency with a mixture of dioxan and water is lower than that with the scintillator in toluene alone; the original efficiency can, however, be obtained by the addition of naphthalene.

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As it is often necessary to assay aqueous solutions, naphthalene was added to the scintillator solution for optimal efficiency. To ascertain whether naphthalene could compensate for the small decrease in efficiency caused by the presence of Thixcin, the efficiencies of the scintillators for counting a tritium standard were determined; the results are shown in Table I.

TABLE I

EFFICIENCY OF COUNTING A TRITIUM STANDARD IN A ONE-CHANNEL LIQUID-SCINTILLATION APPARATUS (NUCLEAR ENTERPRISES (GB) LTD.) WITH DIFFERENT LIQUID SCINTILLATORS

		Counting efficiency (background 60 c.p.m.)	
	Liquid scintillator	not de-oxygenated, %	de-oxygenated for 5 minutes with argon, %
I.	4 g of PPO (2,5-diphenyloxazole), 0·1 g of POPOP (1,4-di-[2-(5-phenyloxazoyl)] benzene) and 70 g of naphthalene per litre dissolved in toluene ..	14·1	16·0
II.	4 g of PPO, 0·1 g of POPOP, 70 g of naphthalene and 25 g of Thixcin per litre dissolved in toluene ..	13·9	15·9
III.	4 g of PPO, 0·1 g of POPOP and 25 g of Thixcin per litre dissolved in toluene	13·5	15·6

It can be seen from Table I that the addition of naphthalene to the Thixcin gel results in the formation of a transparent gel that has the same efficiency as the liquid scintillator. Liquid scintillator I can be used for water and organic fluids and liquid scintillator II for the counting of particles—by applying simple corrections it is possible to achieve the same counting efficiency for both.

Anhydrous sodium sulphate—The tissue must be dehydrated before dispersion in the Thixcin gel for radioassay. This may be done by freeze-drying or by the use of anhydrous sodium sulphate. Since freeze-drying requires extensive laboratory equipment, which was not available, the tissues were dried with sodium sulphate. It was therefore necessary to investigate the influence of the latter on the scintillator solution. Its quenching effect should be minimal to avoid extensive corrections. This was examined in an experiment with anhydrous sodium sulphate, anhydrous sodium sulphate *plus* water (4 + 1) and anhydrous sodium sulphate *plus* water (2 + 1). In each test 10 ml of scintillator gel and 50 μl of a tritium standard solution with different amounts of the sodium sulphate mixtures were counted. From Table II it can be seen that the quenching effect or light absorption is negligible in all mixtures—in concentrations up to 200 mg per 10 ml of liquid scintillator. The sodium sulphate becomes nearly transparent in the gel.

TABLE II

TRITIUM QUENCHING VALUES FOR SODIUM SULPHATE

Amount of sodium sulphate added to 10 ml of liquid scintillator II, mg	Relative counting rate		
	No water	20% of water	40% of water
0	100	100	100
25	101	101	101
50	101	98	101
75	100	100	99
100	101	96	97
125	100	98	97
150	97	98	97
200	99	91	95

The sodium sulphate gel, however, has a phosphorescence that takes much longer to fade than the 10 minutes required for the phosphorescence of an ordinary liquid scintillator to reach the background value.[14] The decline in phosphorescence of a suspension of 100 mg of anhydrous sodium sulphate dispersed in 10 ml of scintillator gel is shown in Fig. 1.

The phosphorescence decreases to the normal background value within approximately 2 hours. The phosphorescence of dried sodium sulphate *plus* water, in the proportions (2 + 1) and (4 + 1), decreases in a similar way.

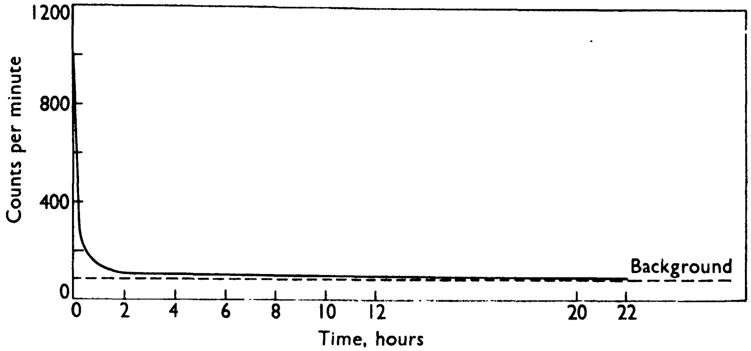

Fig. 1. Decline of phosphorescence in 100 mg of anhydrous sodium sulphate suspended in 10 ml of liquid scintillator II

Corrections for quenching and colour of solution—On measuring ionising radiations in tissue in a liquid-scintillation apparatus several factors influence the results, *e.g.*, ordinary quenching and the colouring matter in the tissue. Correction for these factors can be made by the introduction of an internal standard. The correction factor, k, is obtained by comparing the count rate for the standard solution in the liquid scintillator alone with that for the standard in the liquid scintillator containing the tissue suspension. Thus the correction factor is given by the equation—

$$k = \frac{s}{x - c}$$

where s = the count of the standard,
x = the count of the tissue *plus* standard and
c = the count of the tissue alone.

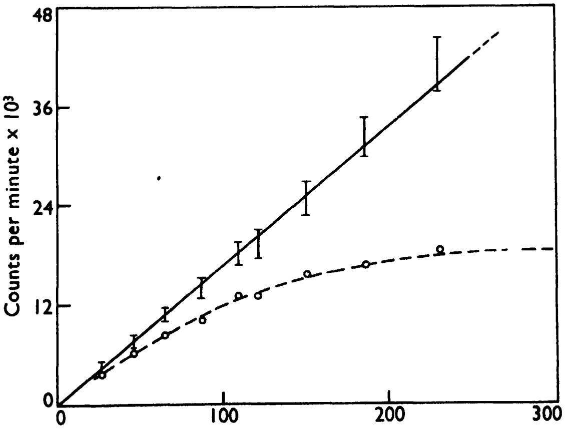

Fig. 2. Relationship between corrected (full line) and uncorrected (broken line) values, in counts per minute, for tritium-labelled vitamin A in liver tissue by the internal standard method

The true count for the tissue is then obtained by multiplying the observed count of the tissue fraction by the correction factor. Fig. 2 shows the relation between uncorrected and corrected values for tritium-labelled vitamin A in liver tissue. The latter values show a direct relationship with the amounts of tissue taken for assay. The tissue was ground with 4 parts of dried sodium sulphate, because it was found that the correction factor for this mixture was approximately one third of that for the (1 + 2) mixture.

PREPARATION OF SAMPLE—

The weighed tissue sample is homogenised in a Griffith tube and then ground with 4 parts of anhydrous sodium sulphate (Pharmacopoea Danica 1948). To ensure uniform mixing, the powder is moistened with light petroleum, which does not quench the light emitted from the liquid scintillator and in which vitamin A is soluble. The mixture is stirred until it is again dry. Material with a high connective tissue content, *e.g.*, lung and muscle tissue, is initially treated with trypsin for one hour at 37° C. Trypsin does not influence the measurements. Faeces are not treated in a Griffith tube, since vitamin A and its decomposition products are free in the faeces and not enclosed by cells.

RANGE OF ERROR, EVALUATION, ETC.—

The coefficient of variation for the radioassay of liver tissue is about 6 per cent. (see Table III) and this variation also applies to other tissues and faeces as well as to the background. As the final result obtained is the difference between two measurements (tissue *minus* background) both with a coefficient of variation of less than 6 per cent., the coefficient of variation of the final result is 8·5 per cent.

As the vitamin-A activity is concentrated in certain tissues, other tissues contain little or no activity. If the count rate for a 0·1-g mixture of a tissue sample is within ±6 per cent. of the value for corresponding tissue from a control animal, it is presumed that the tissue does not contain radioactivity.

Faeces have a high background count as well as a high quenching factor. Daily measurements on faeces are therefore taken during the period of excretion of tritium, and the results are then compared with background results from the period before the animal was dosed with tritium or with the average of the measurements lying within ±6 per cent. of the level found in the faeces after tritium excretion had ceased. The results are corrected for daily variation by means of the daily background count when this count is above or below the average count for the period by ±6 per cent.

TABLE III

STANDARD DEVIATION OF RESULTS ON LIVER TISSUE

Weight of tissue, mg	Count rate, c.p.m.	k	C_k	C_k per 20 mg	$X - \bar{x}$	$(X - \bar{x})^2$
19·7	284	1·60	455	463	+27	729
20·4	297	1·55	460	450	+14	196
20·9	324	1·48	480	459	+23	529
20·0	277	1·45	402	402	−34	1156
20·0	280	1·45	406	406	−30	900
21·0	305	1·48	451	430	− 6	36
20·2	292	1·51	442	437	+ 1	1
20·7	291	1·68	488	471	+35	1225
20·1	280	1·57	439	436	0	0
20·8	292	1·45	424	408	−28	784
			Total ..	4362	Total ..	5556

$$\text{Standard deviation} = \sqrt{\frac{5556}{9}} = 24{\cdot}8$$

A comparison of the sodium sulphate method with the standard method for determining vitamin A (saponification and shaking with an organic liquid) was carried out on certain tissues taken from animals dosed with 10 μC of tritium-labelled vitamin A by measuring the tritium contents. This preparation was examined by paper chromatography to decide whether or not the activity originated from vitamin A. The tissues and the blood were chromatographed on paper in accordance with the technique described by Christensen, Engelund, Handler and Terp.[15] Only the homogenates of the tissue showed a small labelled zone with an R_F value of 0·66 (the R_F value of vitamin A). The results of the comparison are shown in Table IV.

The sodium sulphate method has also been used in other experiments in which the rats were dosed with only 2 µC of tritium-labelled vitamin A per rat.

PROCEDURE FOR SOFT TISSUES—

The organ is removed and weighed, and then ground in a Griffith tube. This tube is normally used for mincing tuberculous materials. The ground tissue is weighed, and mixed with 4 parts of anhydrous sodium sulphate; the sodium sulphate is added gradually. The mixture is then moistened with light petroleum, and stirred until dry; the moistening and stirring are repeated. An aliquot is taken and is moistened with light petroleum and stirred until dry.

A 0·100-g (±10 per cent.) portion of the resulting dry powder is weighed in a measuring cell, 10 ml of liquid scintillator II are added, and the mixture is shaken vigorously for 15 seconds only, care being taken to avoid incorporating air in the suspension, otherwise the suspension may become white and opaque.

TABLE IV

COMPARISON OF THE SODIUM SULPHATE AND THE STANDARD METHODS, BY MEASURING THE TRITIUM ACTIVITY OF CERTAIN TISSUES*

	Liver		Muscle		Blood	
	Count rate, c.p.m. per g	Average, c.p.m. per g	Count rate, c.p.m. per g	Average, c.p.m. per g	Count rate, c.p.m. per g	Average, c.p.m. per g
Assay on unsaponifiable matter	863,000, 835,000	849,000	349,000, 343,000	346,000	17,970, 16,910	17,440
Sodium sulphate method	820,000, 856,000	838,000	354,500, 350,000	352,250	17,100, 18,300	17,700

* It was ascertained by paper chromatography that the activity originated from vitamin A.

TABLE V

MEASUREMENT OF TRITIUM ACTIVITY IN MUSCLE

Weight of muscle = 4·70 g; s = 13,000 c.p.m.

	Test			Control		
	906·9 801·8	832·3 731·6	274·1 173·6	586·8 484·8	435·5 334·0	364·0 263·8
Weight of mixture, mg	105·1	100·7	100·5	102·0	101·5	100·2
Weight of tissue corresponding to weight of mixture, mg	21·0	20·1	20·1	20·4	20·3	20·0
c Counts	10^4	10^4	10^4	3430	3380	3492
c Minutes	6·80	7·09	6·90	20·00	20·00	20·00
c c.p.m.	1470	1410	1448	171	169	175
x Counts	2×10^4	2×10^4	2×10^4	2×10^4	2×10^4	2×10^4
x Minutes	1·60	1·63	1·56	2·00	1·85	1·81
x c.p.m.	12,500	12,250	12,800	10,000	10,800	11,000
$k = \frac{s}{x - c}$	1·23	1·25	1·19	1·37	1·27	1·25
$k \times c$	1810	1760	1720	234	214	219
$k \times c$ per 20 mg	1720	1750	1710	230	211	219
Average c.p.m. per 20 mg		1725			220	

$$\text{Tritium activity in the muscle} = \frac{(1725 - 220) \times 4{\cdot}70}{0{\cdot}02} = 354{,}000 \text{ c.p.m.}$$

The sample is left in darkness until the next day, and then placed in the apparatus in darkness for 10 minutes and then counted (c counts per minute). When this count is completed, 25 µl of a tritium standard solution (s counts per minute) are added in red light. The cell is shaken for 15 seconds, and then left in darkness for approximately 2 hours. A further count is then carried out as before (x counts per minute). The results are treated as shown in Tables V and VI, and the correction factor is calculated. The count rate for the powder (c) is corrected for quenching by multiplying it by the correction factor k (k × c), and the result is then converted to a corrected count for exactly 20·0 mg of tissue. The average of several measurements is calculated. The final result—the total activity in the tissue—is calculated by multiplying the difference between the count rates for the sample and control by the weight of the organ in grams and dividing the result by 0·02.

PROCEDURE FOR BLOOD—

Blood is treated in the same manner as described above for soft tissues.

TABLE VI

MEASUREMENT OF TRITIUM ACTIVITY IN LIVER

Weight of liver = 6·20 g; s = 13,000 c.p.m.

	Test			Control		
	904·4 803·0	837·9 731·5	375·1 274·4	586·8 486·6	437·4 335·2	367·5 264·6
Weight of mixture, mg	101·4	106·4	100·7	100·2	102·2	102·9
Weight of tissue corresponding to weight of mixture, mg	20·3	21·3	20·1	20·0	20·4	20·6
c Counts	4018	4245	4878	4358	4178	4400
c Minutes	20·00	20·00	20·00	20·00	20·00	20·00
c c.p.m.	201	212	244	218	209	220
x Counts	2×10^4	2×10^4	2×10^4	2×10^4	2×10^4	2×10^4
x Minutes	2·48	2·53	2·43	2·54	2·48	2·43
x c.p.m.	8060	7912	8200	7880	8060	8200
$k = \frac{s}{x - c}$	1·67	1·71	1·65	1·72	1·68	1·65
k × c	336	362	405	375	354	364
k × c per 20 mg	331	340	400	375	345	354
Average c.p.m. per 20 mg		357*			358	

* Since 357 c.p.m. is within the limits of 358 ± 21 c.p.m., it is assumed that there is no tritium activity in the liver.

PROCEDURE FOR ORGANS RICH IN CONNECTIVE TISSUE—

The organ is cut into small pieces, placed in a Griffith tube, and then treated with trypsin (approximately 5 mg per g of tissue) at 37° C for 1 hour. The procedure is continued as described for soft tissues. Table VII shows the result of measurements on different tissues.

PROCEDURE FOR FAECES—

Faeces are weighed and ground in a mortar with 4 parts of anhydrous sodium sulphate. The procedure is continued as described above.

The final result—the activity of faeces over a long period—is obtained from the daily count for 20·0 mg of faeces corrected for daily variation, *e.g.*, of the apparatus, by means of the daily background count when it is above or below the average background count of the period by ±6 per cent. (see Table VIII).

The activity of the total daily faecal output is obtained by multiplying the difference between the daily corrected count and the average background count for the period by the total weight of faeces in grams, and dividing the result by 0·02.

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TABLE VII

DETERMINATION OF TRITIUM ACTIVITY, AS TRITIUM-LABELLED VITAMIN A, IN VARIOUS TISSUES

Tissue	Weight of tissue, mg	Count rate, c.p.m.	k	c.p.m. × k	$\frac{\text{c.p.m.} \times k}{\text{per 20 mg}} = C_k$	$C_k - C_b$	Weight of organ, g	Total c.p.m.
Rat 2 (*test*)—								
Liver	20·4	209	1·57	328	322	129	6·7	$\frac{129 \times 6{\cdot}7}{0{\cdot}02} = 43{,}200$
Adrenal gland.. ..	20·9	244	1·69	412	394	0	0·1	0
Lung	20·7	77	2·63	202	196	0	1·4	0
Kidney	20·8	113	1·67	188	181	0	1·4	0
Muscle	21·1	107	1·31	141	134	0	8·6	0
Blood	19·4	56	3·69	206	213	21	1·0	$\frac{21 \times 1{\cdot}0}{0{\cdot}02} = 1050$ (per g)
Rat 3 (*control*)—								
Liver	20·4	123	1·60	196	193*			
Adrenal gland.. ..	20·0	237	1·65	392	392*			
Lung	19·3	78	2·69	207	215*			
Kidney	20·2	116	1·56	181	180*			
Muscle	20·7	114	1·21	138	134*			
Blood	20·6	54	3·66	198	192*			

* These are the values of C_b.

TABLE VIII

MEASUREMENT OF TRITIUM-ACTIVITY IN FAECES (AN EXTRACT FROM A RECORD BOOK FOR 5 DAYS OF A PERIOD OF 16 DAYS)

Date	Weight of faeces, mg	Count rate, c.p.m.	k	c.p.m. × k	c.p.m. × k per 20 mg	f*	$I = \frac{\text{c.p.m.} \times k}{\text{per 20 mg}} \times f^*$	I − g′	Weight of faeces, g	Total c.p.m.
Rat 2 (*test*)—										
8/11	20·3	394	5·32	2090	2060	—	2060	1295	0·13	$\frac{1295 \times 0{\cdot}13}{0{\cdot}02} = 8400$
9/11	20·0	294	4·27	1255	1255	—	1255	490	0·58	$\frac{490 \times 0{\cdot}58}{0{\cdot}02} = 14{,}200$
10/11	20·3	187	3·84	725	712	—	712	0	0·55	0
11/11	20·6	244	4·40	1070	1040	—	832	67	0·70	$\frac{67 \times 0{\cdot}70}{0{\cdot}02} = 2345$
12/11	20·4	216	3·80	821	805	—	805	0	1·00	0
							g′ = 765 ± 46			
Rat 3 (*control*)—										
8/11	19·8	204	4·29	875	884	1·0				
9/11	21·3	210	4·55	955	896	1·0				
10/11	20·3	146	6·66	971	958	1·0				
11/11	20·3	200	5·88	1176	1158	$\frac{905}{1158} = 0{\cdot}8$				
12/11	19·9	208	3·93	906	911	1·0				
					g″ = 905 ± 54					

* f = correction factor for daily variation. g′ = average for the entire period *minus* first 5 days. g″ = average for the entire period.

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The Determination of Strontium in Tap-water by X-ray Fluorescence Spectrometry

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A method is described for determining natural strontium in tap-water by X-ray fluorescence analysis as a preliminary step to the determination of the strontium-90 content. Calcium and strontium are co-precipitated as oxalates and converted to nitrates. A known amount of rubidium is added as internal standard and the ratio of the intensities of the strontium Kα and rubidium Kα lines measured. The rubidium internal standard compensates for the absorption effect of the varying concentration of calcium present in tap-waters, and a linear relationship exists between the intensity ratio of the strontium and rubidium Kα radiation and the weight ratio of strontium to rubidium in the prepared sample.

In determining the radioactive-strontium content of tap-water it is necessary to know the amount of natural strontium present in the sample as this is often comparable with that of the added carrier. The distribution of this element in tap-water usually varies between 0·02 and 0·4 mg per litre, according to locality; examples are shown in Table I. The calcium content is considerably higher and, expressed as a calcium - strontium ratio, varies from 200 to 600. Until recently the total strontium was determined by a flame spectrophotometric method usually involving preliminary removal of calcium. Experiments in which an X-ray fluorescence technique was used suggested that a simpler method could be employed, with rubidium as internal standard, which compensated for the absorption effect due to the variable concentration of calcium in water samples.

EXPERIMENTAL

APPARATUS—

The X-ray fluorescence instrument used was the Research and Control Instruments Ltd. total-vacuum spectrometer. In this equipment the specimen is normally carried in an aluminium cup that has a Mylar-film base through which the primary beam of X-rays irradiates the material. In the proposed method of sample preparation drops of solution are evaporated on to the surface of the Mylar exposed directly to radiation, producing a thin coherent film of residue extending over the whole area irradiated by the primary X-ray beam. This reduces the effect of matrix absorption and increases the ratio of peak to background intensity. The film of residue should be centrally placed in order to obtain good reproducibility. Under these conditions, 0·5 μg of strontium on the film can be measured.

Operating conditions for the instrument are a tungsten target, lithium fluoride crystal analyser and scintillation counter detector. The target potential is 40 kV and the beam current is 20 mA. Both sample and spectrometer chambers are evacuated. Strontium Kα and rubidium Kα radiations have wavelengths of 0·875 Å and 0·926 Å, respectively, the theoretical goniometer settings being 25·09° and 26·58°; these are determined experimentally at the beginning of the analysis of a batch of samples. The background is level, and only one background measurement is made at 25·6°. There are no interfering lines.

PROCEDURE—

An aliquot of the tap-water sample, preferably containing about 20 to 30 μg of strontium, is concentrated to 100 ml. Twenty millilitres of 8 per cent. w/v oxalic acid are added and the solution is adjusted to pH 4 by adding ammonia solution. After the solution has been set aside at 60° C for 1 hour, the precipitate of strontium and calcium oxalates is collected with the aid of a filter stick, and the paper is ignited in a silica dish at 500° C. The resulting carbonates are dissolved in 30 per cent. v/v nitric acid, and the solution is evaporated to dryness.

The residue of nitrates is dissolved in 1 ml of rubidium chloride solution containing 200 µg of rubidium, and two drops of 1/60,000 solution of an anionic wetting agent are added to reduce surface tension. Two drops of the prepared sample solution are then placed on the Mylar-film base of an upturned specimen container, care being taken to ensure that the drops are centrally placed and do not spread to the edge of the film. The cups are placed, still upside down, in a vacuum desiccator. The round spot of liquid immediately freezes and then slowly evaporates over a period of 20 minutes. All the sample material should be within the area irradiated by X-rays, and this can be achieved by the control of the amount of wetting agent added and the number of drops of sample solution taken. After they have been dried, the specimen holders are placed in the instrument, which is then evacuated; this keeps the residue dry and reduces the background radiation due to air scatter.

Standards are prepared by adding suitable volumes of standard strontium nitrate solution to 1 ml of rubidium chloride solution containing 200 µg of rubidium, evaporating to 1 ml, and treating as described in the preceding paragraph.

TABLE I

DISTRIBUTION OF STRONTIUM IN TAP-WATER

Locality	Strontium content, mg per litre	Calcium content, mg per litre	Ratio of calcium to strontium
Belfast	0·005	1·1	220
Loch Lomond (surface water) ..	0·032	4·8	150
Exeter	0·049	25·3	516
Norwich	0·420	97·4	232

TABLE II

DETERMINATION OF STRONTIUM IN WATERS

Strontium added, µg	Strontium found, µg	Ratio of calcium to strontium
Simulated tap-water—		
5	4·5	50
20	18·3	50
30	32·0	50
63	64·0	260
83	81·2	400
Tap-water with added strontium—		
20	21·4	580
5	4·6	500

TABLE III

DETERMINATION OF LOW LEVELS OF STRONTIUM

Strontium content, µg per ml	Rubidium content, µg per ml	Background rate, pulses per second	Net Rb Kα rate (A), pulses per second	Net Sr Kα rate (B), pulses per second	Ratio $\frac{B}{A}$	Strontium found (calculated by comparison with standard), µg
1·7	200	42·4	1850	15·4	0·0083	1·8
0·8	200	38·1	1948	6·76	0·0035	0·8
0·4*	200	35·8	1638	2·05	0·0013	0·3
83·0	200	65·3	321·3	119·3	0·371	—

* The coefficient of variation, calculated from the number of pulses counted, was 15 per cent.

The intensities of the background and of the Kα radiations of strontium and rubidium are measured by counting over duplicate periods of 100 seconds at each angular position. Although the calibration curve is linear, it is the practice in this laboratory to include one standard with each daily batch of tap-water samples, the strontium concentrations being calculated from this standard. This compensates for any slight changes in instrumental sensitivity at the goniometer settings used, and provides a check on the functioning of the scaling and timing electronics. It has been found experimentally that a change of 1 per cent. in the X-ray tube potential causes a change of 0·8 per cent. in peak intensity ratio. During several months of intermittent work the calibration constants have not significantly altered.

Results

The net peak ratio of strontium Kα to rubidium Kα has been determined for a number of solutions of different strontium and calcium concentrations. A linear relation has been found to exist between the peak ratio and strontium concentrations from 50 to 1000 μg per ml with calcium - strontium ratios ranging from 0 to 400. The relation for this instrument can be expressed as—

$$\frac{\text{Sr K}\alpha}{\text{Rb K}\alpha} = 0{\cdot}96 \times \frac{\text{Concentration of strontium}}{\text{Concentration of rubidium}}$$

A number of simulated tap-waters containing known amounts of strontium and also natural waters to which strontium had been added have been analysed; some of these results are shown in Table II. It is possible to determine 5 μg of strontium in a sample with reasonable precision. In such instances, the 1 ml of solution, obtained after adding the rubidium chloride, is concentrated to about 0·3 ml before the spot on the film is prepared. The ultimate sensitivity of the method depends on the ratio of peak to background and, in order to determine the lower limits of measurement, standards were prepared containing 1·6, 0·8 and 0·4 μg of strontium, each with a calcium - strontium ratio of 400. These were concentrated to about 0·2 ml and 4 drops were taken; thus almost all the strontium was placed on the film. In Table III are shown the count rates and ratios obtained over extended counting periods of 800 seconds. The peak ratios are compared with that for the high level standard to determine the amount of strontium. The results indicate that the determination of 0·5 μg is feasible by this method. In these conditions a useful reduction of background can be achieved by lining the inside of the specimen holder with lead to reduce the scatter of white radiation from the aluminium walls.

The precision depends on counting statistics and the reproducibility of the sample preparation technique. With counting periods of 100 seconds, a coefficient of variation of better than 5 per cent. at the 40 μg level was obtained, which improved to 1·8 per cent. at 275 μg of strontium.

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The Micro-determination of Hydrazine Salts and Certain Derivatives

By M. Z. BARAKAT and MONIER SHAKER

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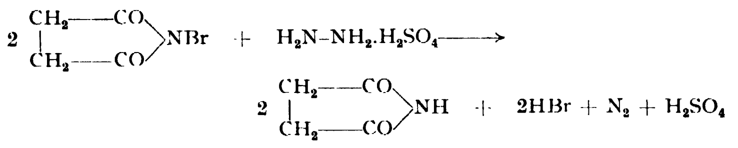
In the presence of dilute sulphuric acid, *N*-bromosuccinimide readily and quantitatively oxidises aqueous solutions of hydrazine salts and certain derivatives. The reaction takes place at room temperature, and *N*-bromosuccinimide is irreversibly reduced to succinimide. The mechanism of the reaction is discussed. The determination of hydrazine sulphate, phenylhydrazine hydrochloride and phenylhydrazine-*p*-sulphonic acid by titration with standard *N*-bromosuccinimide solution is described; the experimental error does not exceed ±2 per cent. Results are reported for comparative analysis of hydrazine sulphate and phenylhydrazine hydrochloride by the proposed method and by the generally accepted potassium iodate method.

Various titrimetric,[1,2] colorimetric[3] and potentiometric[4] methods have been described for determining hydrazine salts, and the oxidation of hydrazine[5,6,7] has been widely used as the basis of techniques for its measurement.

The titrimetric methods suffer from certain defects, *e.g.*, oxidation of hydrazine in alkaline solution with potassium permanganate[8] gives inaccurate results in the absence of barium and telluric acid. The end-point in the chloramine B method[9] is indistinct; in the presence of an excess of potassium bromide the end-point is sharp and accurate results can be obtained. There is some doubt about the reliability of the potassium iodate method.[5,10]

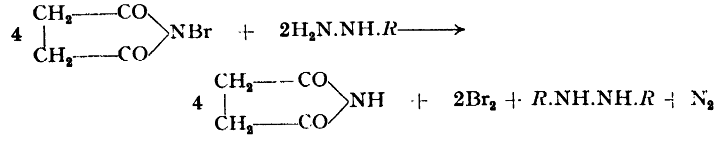
The investigation described here deals with the micro-determination of hydrazine salts, *e.g.*, hydrazine sulphate and certain derivatives such as phenylhydrazine hydrochloride and phenylhydrazine-*p*-sulphonic acid, by the use of *N*-bromosuccinimide. The method is based on the fact that *N*-bromosuccinimide[11] readily and quantitatively oxidises an aqueous solution of hydrazine sulphate and is itself reduced to succinimide, with the evolution of nitrogen and the formation of hydrogen bromide, as represented by the equation—



$$2\left.\begin{matrix}CH_2\text{—}CO\\ |\qquad\\ CH_2\text{—}CO\end{matrix}\right\rangle NBr + H_2N\text{-}NH_2.H_2SO_4 \longrightarrow 2\left.\begin{matrix}CH_2\text{—}CO\\ |\qquad\\ CH_2\text{—}CO\end{matrix}\right\rangle NH + 2HBr + N_2 + H_2SO_4$$

The reaction proceeds quantitatively in aqueous solution at room temperature in the ratio of two molecules of *N*-bromosuccinimide to one molecule of hydrazine sulphate. Effervescence owing to evolution of nitrogen is perceptible. Succinimide has been isolated from the reaction mixture and identified by melting-point and mixed melting-point determinations. The presence of hydrogen bromide and sulphuric acid in the reaction mixture has been confirmed.

Similarly, *N*-bromosuccinimide is quantitatively and rapidly reduced to succinimide by phenylhydrazine[12] or phenylhydrazine-*p*-sulphonic acid at room temperature, in accordance with the equation—



$$4\left.\begin{matrix}CH_2\text{—}CO\\ |\qquad\\ CH_2\text{—}CO\end{matrix}\right\rangle NBr + 2H_2N.NH.R \longrightarrow 4\left.\begin{matrix}CH_2\text{—}CO\\ |\qquad\\ CH_2\text{—}CO\end{matrix}\right\rangle NH + 2Br_2 + R.NH.NH.R + N_2$$

where R can be C_6H_5– or $HO_3S.C_6H_4$–.

With hydrazine derivatives, *e.g.*, phenylhydrazine, bromine is formed, not hydrogen bromide as is formed with hydrazine sulphate.

N-Bromosuccinimide is a highly selective oxidising agent[13,14] and can decolorise methyl red in aqueous acid medium, but it oxidises hydrazine salts or derivatives preferentially. Until all the hydrazine salt or derivative present has been oxidised, the red colour of the indicator does not disappear. The slightest excess of *N*-bromosuccinimide added after all the hydrazine salt or derivative has been oxidised decolorises the methyl red. Methyl red has been used previously as an indicator in titrations involving the use of bromine[15] and *N*-bromosuccinimide.[16]

Comparative determinations of hydrazine salts have been carried out by the proposed method and the most generally accepted potassium iodate method. *N*-Bromosuccinimide is analogous to potassium iodate; it acts as an oxidising agent, but is better than iodate in that it can be used for determining extremely small amounts of hydrazine salts or derivatives. The potassium iodate method cannot be used for determining amounts of hydrazine salts less than 30 mg, whereas the proposed method will determine amounts as low as 500 µg.

EXPERIMENTAL

REACTION BETWEEN *N*-BROMOSUCCINIMIDE AND HYDRAZINE SULPHATE—

A 1·3013-g portion of hydrazine sulphate (0·01 mole) was dissolved in 20 ml of distilled water, and 3·56 g of *N*-bromosuccinimide (0·02 mole) were dissolved in 200 ml of hot distilled water. When the *N*-bromosuccinimide solution was cool, it was added gradually, with shaking, to the cold hydrazine sulphate solution. During the addition a strong effervescence was observed owing to evolution of nitrogen. When the bubbles of gas had ceased, the reaction mixture was a colourless solution.

The presence of hydrobromic acid was established by treating 10 ml of the colourless solution with nitric acid and 10 per cent. silver nitrate solution; a yellowish white precipitate of silver bromide was deposited.

The presence of sulphuric acid was confirmed by treating 10 ml of the colourless solution with hydrochloric acid and 10 per cent. barium chloride solution; a white precipitate of barium sulphate was observed.

Succinimide was isolated by distilling *in vacuo* the remaining 200 ml of the colourless solution and crystallising the solid residue from benzene. Melting-point and mixed melting-point determinations with an authentic sample showed that the colourless crystals obtained were succinimide.

VALIDITY OF THE REACTION FOR QUANTITATIVE DETERMINATION—

Before applying the reaction to the determination of hydrazine salts or derivatives in test solutions, we decided to verify quantitatively the reaction between *N*-bromosuccinimide and hydrazine sulphate. An accurately measured volume of solution containing 0·13013 g (1 millimole) of hydrazine sulphate per 100 ml was placed in a 25-ml conical flask, and an equal volume of 10 per cent. v/v sulphuric acid and 2 drops of 0·04 per cent. methyl red indicator solution in 95 per cent. ethanol were added. The mixture was titrated with 0·1 per cent. w/v *N*-bromosuccinimide solution, added dropwise from a microburette, with continuous shaking after each addition. When the red colour of the indicator faded, another drop was added, and the titration was continued until the red colour completely disappeared. A similar series of experiments was carried out with *N*-bromosuccinimide solution containing twice the number of molecules of solute as in the first solution. It was found that the reaction was stoicheiometric in the presence of dilute sulphuric acid at room temperature; the results were—

Volume of hydrazine sulphate solution (1 mmole per 100 ml), ml	10	5	4	3	2	1
Titre of *N*-bromosuccinimide (1 mmole per 100 ml), ml	20·25	10·15	8·12	6·18	4·11	2·06
Titre of *N*-bromosuccinimide (2 mmole per 100 ml), ml	10·13	5·10	4·09	3·08	2·05	1·02

The equimolecular solutions of hydrazine sulphate and *N*-bromosuccinimide (freshly prepared) were then diluted ten-fold with distilled water in 100-ml calibrated flasks. The diluted solutions were titrated as before and gave reproducible results.

METHOD

REAGENTS—

Methyl red solution, 0·04 *per cent. w/v in* 95 *per cent. ethanol.*

Dilute sulphuric acid, 10 *per cent. v/v.*

Dilute hydrochloric acid, 10 *per cent. v/v.*

N-*Bromosuccinimide solution,* 0·1 *per cent. w/v, aqueous*—This solution should be freshly prepared before use.

PROCEDURE—

To an accurately measured volume, *e.g.*, 5 ml, of the hydrazine sulphate solution in a 50-ml conical flask add an equal volume of dilute sulphuric acid and 5 drops of methyl red indicator solution. Titrate the mixture with 0·1 per cent. w/v *N*-bromosuccinimide solution, added dropwise from a microburette graduated at 0·01-ml intervals, with continuous shaking. The end-point is reached when the last drop of titrant discharges the red colour. Calculate the hydrazine sulphate content of the sample solution from the expression—

$$\text{Hydrazine sulphate present, mg or } \mu g = 130{\cdot}13\, VC/178$$

where V is the titre of *N*-bromosuccinimide solution in millilitres and C is the concentration of the *N*-bromosuccinimide solution in mg or µg per ml.

APPLICATIONS OF THE METHOD

DETERMINATION OF HYDRAZINE SULPHATE—

A 0·5 per cent. stock solution of hydrazine sulphate was prepared by dissolving 0·5 g of pure hydrazine sulphate, obtained from E Merck A.G., Darmstadt, Germany, in distilled water, and the volume was adjusted to 100 ml with distilled water in a calibrated flask. Solutions containing 0·4, 0·3, 0·2 and 0·1 per cent. of hydrazine sulphate were prepared by suitable dilutions of portions of this solution. The hydrazine sulphate contents of these solutions were then determined by the proposed method; the results are shown in Table I.

TABLE I

RECOVERY OF HYDRAZINE SULPHATE BY THE PROPOSED METHOD

Concentration of hydrazine sulphate solution, % w/v	Volume of solution used, ml	Hydrazine sulphate content, mg	Titre of 0·1 per cent. w/v *N*-bromosuccinimide solution, ml	Hydrazine sulphate found, mg	Error, %
0·5	1	5	13·55	4·95	1·00
	2	10	27·00	9·87	1·30
	3	15	40·50	14·80	1·33
0·4	1	4	11·05	4·04	1·00
	2	8	22·25	8·13	1·63
0·3	1	3	8·30	3·03	1·00
	2	6	16·55	6·05	0·83
0·2	1	2	5·55	2·03	1·50
	2	4	11·05	4·04	1·00
0·1	1	1	2·70	0·99	1·00
	2	2	5·47	2·00	—
	3	3	8·34	3·05	1·66
	4	4	11·05	4·04	1·00
	5	5	13·80	5·04	0·80
	6	6	16·25	5·94	1·00
	7	7	18·95	6·93	1·00
	8	8	21·70	7·93	0·88
	9	9	24·45	8·94	0·75
	10	10	27·10	9·91	0·90

The 0·1 per cent. hydrazine sulphate solution was then diluted ten-fold with distilled water in a 100-ml calibrated flask and the diluted solution (0·01 per cent.) was titrated with 0·05 per cent. w/v *N*-bromosuccinimide solution; the results are shown in Table II.

TABLE II

MICRO-DETERMINATION OF HYDRAZINE SULPHATE BY THE PROPOSED METHOD

Volume of hydrazine sulphate solution used, ml	Hydrazine sulphate present, µg	Titre of 0·05 per cent. *N*-bromosuccinimide solution, ml	Hydrazine sulphate found, µg	Error, %
10	1000	5·45	996·26	0·37
9	900	4·90	895·72	0·47
8	800	4·35	795·18	0·60
7	700	3·80	694·64	0·77
6	600	3·30	603·24	0·52
5	500	2·70	493·56	1·29

The proposed method was compared with the iodate method by titrating a 1 per cent. hydrazine sulphate solution with 0·5 per cent. *N*-bromosuccinimide solution and with 0·025 M potassium iodate; the results are shown in Table III.

TABLE III

COMPARISON OF RESULTS BY PROPOSED AND IODATE METHODS

1 ml of 0·5 per cent. *N*-bromosuccinimide ≡ 1·8275 mg of hydrazine sulphate
1 ml of 0·025 M potassium iodate ≡ 3·253 mg of hydrazine sulphate

		With 0·5 per cent. *N*-bromosuccinimide as titrant			With 0·025 M potassium iodate as titrant		
Volume of 1 per cent. w/v hydrazine sulphate solution taken, ml	Hydrazine sulphate content, mg	Titre, ml	Hydrazine sulphate found, mg	Error, %	Titre, ml	Hydrazine sulphate found mg	Error, %
10	100	55·40	101·24	1·24	30·55	99·38	0·62
9	90	50·10	91·56	1·73	27·60	89·78	0·24
8	80	44·10	80·59	0·74	24·50	79·70	0·43
7	70	38·50	70·36	0·51	21·55	70·10	0·14
6	60	33·10	60·49	0·82	18·40	59·86	0·23
5	50	27·60	50·44	0·88	15·20	49·45	1·10
4	40	22·10	40·39	0·98	12·45	40·50	1·25
3	30	16·60	30·34	1·13	9·35	30·42	1·40
2	20	10·90	19·92	0·40	6·30	20·49	2·45
1	10	5·50	10·05	0·50	2·95	9·60	4·00
0·5	5	2·76	5·04	0·80	1·70	5·53	10·60

DETERMINATION OF PHENYLHYDRAZINE HYDROCHLORIDE—

Before the proposed method was applied to determining phenylhydrazine hydrochloride, it was decided to verify quantitatively the reaction between *N*-bromosuccinimide and phenylhydrazine hydrochloride. It was assumed that one molecule of phenylhydrazine hydrochloride was oxidised by two molecules of *N*-bromosuccinimide. Accordingly, a solution containing 0·1445 g (1 millimole) of phenyhydrazine hydrochloride per 100 ml was titrated with solutions containing 0·178 and 0·356 g (1 and 2 millimoles, respectively) of *N*-bromosuccinimide. It was found that the reaction between phenylhydrazine hydrochloride and *N*-bromosuccinimide was quantitative in the molecular concentrations expected; the results were—

Volume of phenylhydrazine hydrochloride (1 mmole per 100 ml), ml	10	5	4	3	2	1
Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml ..	19·95	9·95	8·05	6·05	3·95	2·05
Titre of *N*-bromosuccinimide solution (2 mmoles per 100 ml), ml ..	10	5	4	3	2	1

A series of experiments was carried out in which a 0·1 per cent. w/v solution of phenylhydrazine hydrochloride was titrated by the proposed method with 0·1 per cent.*N*-bromosuccinimide; the results are shown in Table IV.

TABLE IV

RECOVERY OF PHENYLHYDRAZINE HYDROCHLORIDE BY PROPOSED AND IODATE METHODS

1 ml of 0·1 per cent. *N*-bromosuccinimide ≡ 0·4059 mg of phenylhydrazine hydrochloride
1 ml of 0·025 M potassium iodate ≡ 3·612 mg of phenylhydrazine hydrochloride

	With 0·1 per cent. *N*-bromosuccinimide as titrant			With 0·025 M potassium iodate as titrant		
Phenylhydrazine hydrochloride present, mg	Titre, ml	Phenylhydrazine hydrochloride found, mg	Error, %	Titre, ml	Phenylhydrazine hydrochloride found, mg	Error, %
10	24·45	9·92	0·80	2·45	8·85	11·50
9	22·00	8·93	0·78	2·15	7·77	13·67
8	19·65	7·98	0·25	1·90	6·86	14·25
7	17·03	6·91	1·29	1·70	6·14	12·29
6	14·60	5·93	1·17	1·55	5·60	6·67
5	12·13	4·92	1·60	1·25	4·52	9·60

DETERMINATION OF PHENYLHYDRAZINE-*p*-SULPHONIC ACID—

It was ascertained quantitatively that one molecule of phenylhydrazine-*p*-sulphonic acid was oxidised by two moles of *N*-bromosuccinimide; the results obtained were—

Volume of phenylhydrazine-*p*-sulphonic acid solution (1 mmole per 100 ml), ml	10	5	4	3	2	1
Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml..	20·08	10·05	8·04	6·02	4·05	2·05

A 0·1 per cent. solution of phenylhydrazine-*p*-sulphonic acid was titrated by the proposed method with 0·1 per cent. *N*-bromosuccinimide solution; the results are shown in Table V.

TABLE V

DETERMINATION OF PHENYLHYDRAZINE-*p*-SULPHONIC ACID BY THE PROPOSED METHOD

1 ml of 0·1 per cent. *N*-bromosuccinimide ≡ 0·5284 mg of phenylhydrazine-*p*-sulphonic acid

Volume of phenylhydrazine-*p*-sulphonic acid solution, ml	Phenylhydrazine-*p*-sulphonic acid present, mg	Titre of 0·1 per cent. w/v *N*-bromosuccinimide, ml	Phenylhydrazine-*p*-sulphonic acid found, mg	Error, %
10	10	18·85	9·96	0·40
9	9	17·00	8·98	0·22
8	8	15·20	8·03	0·38
7	7	13·15	6·95	0·71
6	6	11·35	6·00	—
5	5	9·40	4·97	0·60
4	4	7·65	4·04	1·00
3	3	5·70	3·01	0·33
2	2	3·75	1·98	1·00
1	1	1·86	0·98	2·00

DISCUSSION OF RESULTS

The accuracy of the generally accepted potassium iodate method depends mainly on the concentration of hydrazine sulphate in the sample solution.

Results show that the experimental error appears to increase as the concentration of hydrazine sulphate in the sample solution decreases. Moreover, the potassium iodate method cannot be used for determining amounts of hydrazine sulphate less than 30 mg (see Table III); further, it is difficult to detect the end-point when the iodate method is applied to low concentrations of hydrazine salts.

The results in Tables III and IV show that the proposed method is superior to the potassium iodate method in accuracy and sensitivity.

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SHORT PAPERS

Rapid Determination of Iodine-131 in Milk

By A. L. BONI

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EXISTING methods for determining the concentrations of iodine-131 in milk involve either complex chemical separations or lengthy gamma-spectrometric analyses of large-volume samples.

Direct absorption of iodine-131 on anion-exchange resin and subsequent gamma-spectrometric analysis of the resin was found to be quantitative as well as time and labour saving in the routine determination of this radionuclide in milk. The method requires less than 2 hours per 1-gallon sample and a counting time of only 10 minutes to achieve a minimum detection limit of $3{\cdot}5 \times 10^{-15}$ curie per ml. The anion-exchange concentration step completely eliminates the interference of all other fission-product contaminants found in milk except ruthenium-103, ruthenium-106 and zirconium - niobium-95. Routine laboratory experience with the method during a period of 1½ years showed an average recovery of 98 per cent.

METHOD

PREPARATION OF ION-EXCHANGE COLUMN—

Loosely place a small plug of glass-wool in the bottom of a 1½-inch × 4-inch Pyrex-glass column (column with cap is illustrated in Fig. 1). Fill the column with 90-ml of a water slurry of previously washed Dowex 1 × 8 (20 to 50 mesh) anion-exchange resin. After any water present has drained to the level of the resin, place a thin film of glass-wool over the bed. Fit the glass cap lubricated with stopcock grease on to the top of the column. With the bottom stopcock closed, carefully add water through the stopcock in the column cap until the entire space above the resin, including the stopcock, is full. Close the top stopcock. Connect an inverted 1-gallon polythene bottle, with the bottom removed, to the top of the column, and then connect a 4-litre filtering flask to the bottom of the column by means of a glass tube (see Fig. 2).

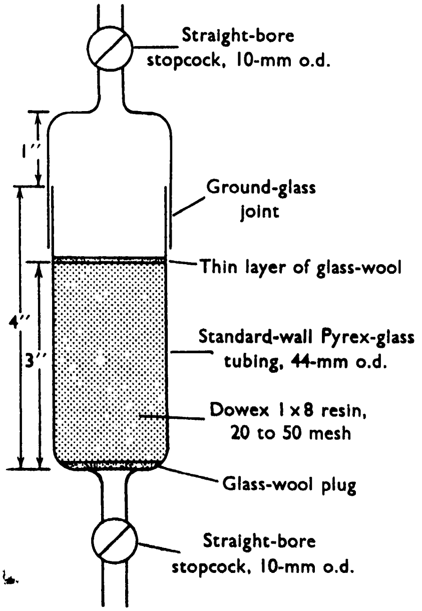
Straight-bore stopcock, 10-mm o.d.
1″
Ground-glass joint
Thin layer of glass-wool
4″
3″
Standard-wall Pyrex-glass tubing, 44-mm o.d.
Dowex 1 x 8 resin, 20 to 50 mesh
Glass-wool plug
Straight-bore stopcock, 10-mm o.d.

Fig. 1. Design of ion-exchange column

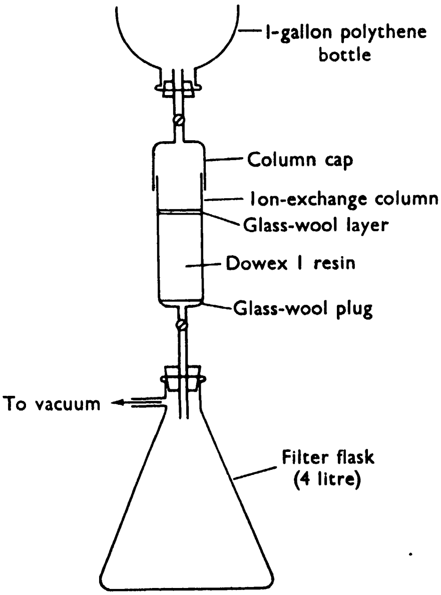

Fig. 2. Design of apparatus

PROCEDURE—

Put a measured ½ gallon of well-mixed fresh milk in the polythene container, and open the top stopcock. Evacuate the filter flask, and then open the bottom stopcock to obtain a flow rate of 30 to 40 ml per minute. Experience has shown that a vacuum is needed only with non-homogenised milk that is several days old or milk containing large amounts of cream. Before the first ½ gallon has drained completely from the polythene container, add a second measured ½ gallon (total volume of milk, 3785 ml). Before the second ½ gallon has drained completely from the polythene container, rinse the container with distilled water, and let the entire liquid volume drain to the level of the resin. (If for any reason the flow through the column has to be stopped during the analysis, always close the bottom stopcock, and never allow the milk to stand over the resin longer than is absolutely necessary, in order to avoid clotting.) Remove the cap from the column, and pass 30 to 40 ml of distilled water through the resin bed. Discard all effluents.

Disconnect the column from the system, invert, and with a stream of air force the resin from the column into a 500-ml 3-inch diameter polythene bottle from which the narrow mouth has been removed. With distilled water, remove any traces of resin remaining in the column, and add the resin and water to the contents of the polythene bottle. Make a slurry of the resin in the bottle with more distilled water, if necessary, in order to mix the resin evenly. Seal the top of the bottle.

COUNTING AND CALCULATIONS—

Place the resin container on top of a thallium-activated 3-inch × 3-inch sodium iodide crystal coupled to a multi-channel gamma spectrometer, and take a 10-minute count. The total count per minute under the iodine-131 0·36-MeV peak must be corrected for background and multiplied by the appropriate conversion factor (6×10^{-12} curie per count per minute). The conversion factor is obtained by counting a known iodine-131 standard in the same geometry as the samples. This figure, divided by the sample volume in millilitres and corrected for recovery by the procedure (98 per cent.), will give the concentration of iodine-131 in curies per millilitre.

$$\text{Concentration of } ^{131}\text{I},\ 10^{-15}\ \text{C per ml} = \text{Conversion factor} \times \text{total count in 10 minutes} \times \frac{1}{3785} \times \frac{1}{0{\cdot}98}.$$

Milk has a high decontamination factor for ruthenium-103, ruthenium-106 and zirconium - niobium-95, but, if detectable amounts of these radionuclides are nevertheless present, the total count under the iodine-131 0·36-MeV peak and the total counts under the peak of these other radionuclides are tabulated from the gamma-ray spectrum. These results, together with the counting time, sample volume and iodine decay factor, are programmed into a computer and solved for the iodine-131 content in curies per millilitre of milk by Compton scatter correction.

DISCUSSION OF THE METHOD

ION EXCHANGE—

Iodine-131, with other anions in milk, is directly absorbed on Dowex 1 × 8 anion-exchange resin.[1] Although some anion-complex formation of ruthenium-103, ruthenium-106 and zirconium - niobium-95 results in their slight concentration, if present, on the resin, the ion-exchange concentration step quantitatively separates iodine-131 from all other principal radionuclides found in milk. Analysis of the resin effluent substantiated that the iodine-131 in milk is free and not bound. Other than iodine-131, the principal radionuclides in milk, ^{89}Sr, ^{90}Sr, ^{137}Cs, ^{140}Ba and ^{40}K, are cations. Since they do not form anion complexes in the milk, they are not concentrated by the anion resin. The various radionuclides reported in milk and their percentage absorption on the anion resin are: ^{131}I, $>$99 per cent.; ^{137}Cs, ^{89}Sr, ^{90}Sr, Ba - ^{140}La, Ce - ^{144}Pr and ^{40}K, $<$1 per cent.; ^{103}Ru and ^{106}Ru, 16 per cent.; Zr - ^{95}Nb, 12 per cent.

The concentration and essentially complete separation of iodine-131 from these other contaminating radionuclides results in a rapid and accurate determination of low levels of the radionuclide in milk.

A column operation is used instead of batchwise mixing, since a much more favourable separation is achieved. Fresh milk and a fast rate of flow are essential, because milk will clog the resin bed if it is allowed to turn sour. Preservatives such as formaldehyde should be avoided, since they complex the iodine and inhibit its absorption on the resin. A flow rate of 30 to 40 ml per minute (*i.e.*, 1 gallon in 1·5 hours) is sufficient. A faster flow rate will cause incomplete absorption of the iodine-131, because insufficient time would be allowed for equilibrium to be reached between the radionuclide and the resin.

The capacity of the column for various amounts of milk can be increased or decreased by changing the length of the resin column proportionately.

COUNTING AND CALCULATIONS—

Because a constant geometry is needed in order to calculate the iodine content from sample to sample on a relative basis, the resin is transferred with water to a 500-ml polythene bottle. The volume of water used to transfer the resin is not critical, since the iodine is not extracted into the water and the resin settles to the bottom of the bottle.

One of the generally accepted methods used for determining quantitatively the concentration of particular isotopes from a gamma-ray spectrum is to subtract the Compton scatter contributions due to other radionuclides from the total count under the photo-peak of the radionuclide of interest.[2] Quantitative analysis by gamma-ray spectrometry becomes more difficult as the number of radionuclides in the sample increases.

Since iodine-131 is completely separated from all other radionuclides except ruthenium-103, ruthenium-106 and zirconium - niobium-95 (which are rarely observed in milk), the counting rate under its main photo-peak (0·36 MeV) is not affected by other radionuclides. The direct application of a conversion factor to this counting rate gives the total amount of iodine-131 present in the volume of milk sampled.

RECOVERY AND DETECTION LIMIT—

The method has yielded an average recovery of 98 per cent. with a standard of activity of 120 × 10^{-15} C per ml over a period of 1½ years; the precision was 17 per cent. (90 per cent. confidence limits). Statistics on the recovery procedure are shown in Fig. 3. The detection limit of the method is limited by the volume of milk (3785 ml), the efficiency and resolution of the sodium iodide crystal detector and the background count.

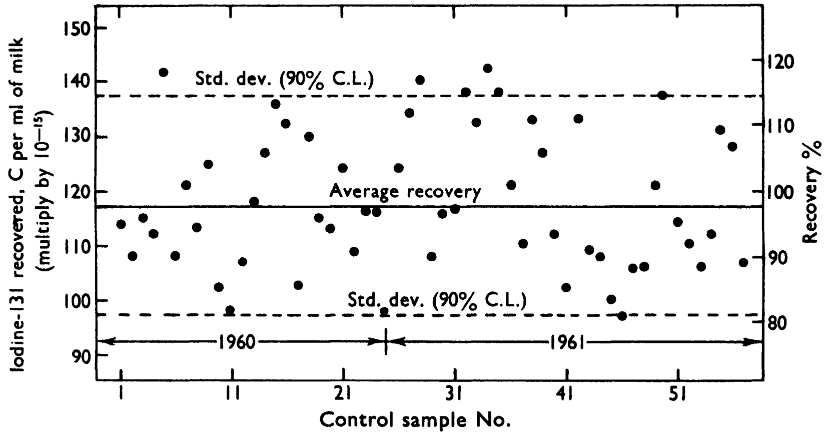

Fig. 3. Recovery statistics (iodine-131 added = 120 × 10^{-15} C per ml of milk)

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The Determination of Lead in Carbon and Low-alloy Steel according to British Standard 1121: Part 41: 1960

By A. CLAASSEN AND L. BASTINGS

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IN the determination of lead in carbon and low-alloy steel as described in the British Standard,[1,2] lead is first precipitated as lead sulphide from a solution of the steel in hydrochloric acid, after the excess of acid has been removed by evaporation "almost to dryness." In this procedure, therefore, no provision is made for a more accurate adjustment of pH before hydrogen sulphide is passed through the solution. The American A.S.T.M. standard,[3] describing practically the same method, specifies the adjustment of pH to 2·0 to 2·5, and states that below pH 1·8 the precipitation of lead sulphide is incomplete and above pH 3·0 co-precipitation of iron occurs.

This led us to investigate the pH values of solutions obtained by carrying out the procedure described in the British Standard. Solutions of 10 g of pure iron (Fisher Combax accelerator) in hydrochloric acid to which 5·0 mg of lead (as a solution of lead chloride) had been added, were treated as described in section 5 of this standard. Before the hydrogen sulphide was passed through the solution, the pH was determined by means of a glass electrode and was found to be between 0·9 and 1·6. Within this range, the pH was dependent on the degree to which the solution had been evaporated; evaporation such that the hot residue was syrupy resulted in the lowest pH value being obtained, and evaporation such that the hot residue was just dry gave the highest pH value. Solutions of pH 0·9 were clear, whereas solutions of pH 1·6 were turbid when cold and hydrolysed appreciably when heated. In the latter solutions, addition of 2 ml of hydrochloric acid, sp.gr. 1·18, as recommended in Note 1 of the British Standard, lowered the pH by about 0·4 unit.

Precipitation of lead in these solutions took place slowly. After hydrogen sulphide had been passed for 20 minutes, as specified, the solutions were filtered, and the amount of lead in the filtrates was determined photometrically with dithizone. The solutions of pH 0·9 contained about 1·2 mg of unprecipitated lead, those of pH 1·3 about 0·25 mg and those of pH 1·8 about 0·06 mg.

When the passage of hydrogen sulphide was continued for 60 minutes instead of the specified 20 minutes, the amount of unprecipitated lead remaining in the solution was smaller; 0·2 mg at pH 0·9 and 0·7 mg at pH 1·6.

It is clear, therefore, that under the conditions of the procedure described in the British Standard, precipitation of lead is more or less incomplete. This is probably the reason that the B.I.S.R.A. Methods of Analysis Committee,[2] in investigating the lower limit of this method, found erratic and low results with amounts of lead below 5 mg.

Obviously it is necessary to precipitate the lead at a higher pH. Increasing the pH by adding ammonia solution incurs difficulties, however, as lumps of ferric hydroxide are formed that redissolve only slowly. With an extremely dilute solution of ammonia this difficulty is minimised, but neutralisation takes a longer time. The formation of lumps of ferric hydroxide was caused by the presence of considerable amounts of tervalent iron formed by aerial oxidation of the iron during evaporation of the solution to dryness. It proved easy to reduce the tervalent iron with ascorbic acid, which can be added in small portions as a powder while the solution is being stirred. The progress of the reduction is indicated by the change in the colour of the solution to light green. In our experiments about 200 mg of ascorbic acid were adequate for complete reduction. Separate experiments showed that even a large excess (1 g) of ascorbic acid does not interfere with the quantitative precipitation of lead, as sulphide, in the pH region 2 to 3. Adjustment of pH can now easily be made, the reduced solution remaining practically clear during the addition of ammonia solution, since ferrous hydroxide only begins to precipitate above pH 5. The measurement of pH with a narrow-range indicator paper is sufficiently accurate.

When hydrogen sulphide is passed into these reduced solutions adjusted to a pH between 2·0 and 2·8, lead sulphide precipitates extremely quickly and soon settles. The filtrates of solutions treated in this way contained less than 10 μg of lead after hydrogen sulphide had been passed through them for 20 minutes.

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A Compact Unit for the Determination of Carbon and Hydrogen by the Večeřa Method

BY D. R. CLIFFORD AND E. A. FRIBBINS

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THE Večeřa[1] method for the micro-determination of carbon and hydrogen has been in use in this laboratory for some time. Knobloch, Knobloch and Mai[2] have published details of an apparatus for the automatic combustion of samples by this method. This Note describes a simple, compact manually-operated apparatus.

The complete unit is shown in Fig. 1. It consists of a base, a metal box containing electrical components, a wooden panel holding the flowmeter, etc., and a chain-driven bunsen burner for burning the sample.

The small electrical furnace for heating the combustion-tube filling is made from a standard 600-watt Nichrome electric-fire element embedded in a cylindrical block of Sindanyo. To accept the element, holes are drilled on a suitable pitch circle so that they "break through" into a 0·44-inch diameter central hole that fits the combustion tube. The whole furnace is mounted in a fabricated stainless-steel pipe "tee." The completed unit is 2·2 inches long and has a diameteer of 1·4 inches. It is supported alongside the top of the instrument case by a Sindanyo panel that serves as a heat insulator. A current setting of 1·1 amps permits the catalyst layer to be maintained at 600° C.

The instrument case houses a 0- to 240-volt Variac transformer, ammeter, fuses and warning light. A subsidiary heater for warming the end of the water-absorption tube is attached to the top of the case, and a slot is cut in the top to accommodate the Večeřa-type[1] carbon dioxide absorption tube.

The wooden panel is bolted to the metal base of the apparatus. It holds a White - Wright type flowmeter,[3] a scavenging U-tube, a flow regulator and a precision pinch cock.

The burner unit consists of a bunsen burner mounted on a carriage that can be moved by operating the chain drive by means of a knob on the front of the apparatus.

Oxygen is supplied from a cylinder via a pressure regulator located at the back of the wooden panel.

The over-all dimensions of the apparatus are: length 23 inches, width 5½ inches, total height 15 inches.

Six to eight determinations per working day are easily achieved by one operator.

We thank Dr. D. Woodcock for his interest and encouragement, Mr. R. C. Perkins for constructing the glassware and Messrs. R. H. Davis and T. Rogers for valuable technical assistance.

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Received *August 10th*, 1962

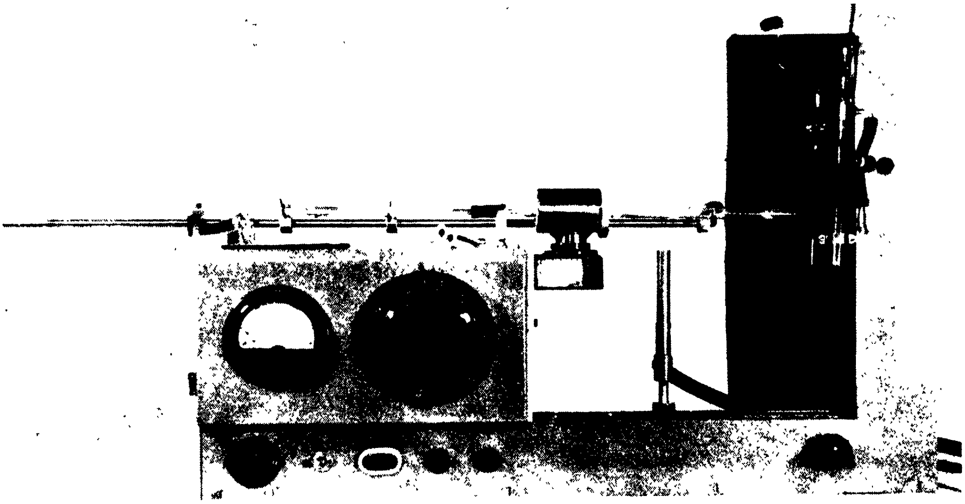

Fig. 1. Complete apparatus

[To face page 68

Book Reviews

THE DITHIOCARBAMATES AND RELATED COMPOUNDS. By G. D. THORN and R. A. LUDWIG. Pp. viii + 298. Amsterdam and New York: Elsevier Publishing Company. 1962. Price Dfl. 20.

The authors describe their work as a "digest of the extensive literature on the dithiocarbamates . . ." and it must be remarked that a vast amount of information has been digested into a readily assimilable form. Although it has the cursory character of a review, each chapter is followed by such a comprehensive list of literature references that the reader may readily trace the details of his own particular interests. These references amount to over 1000, dated up to December, 1960, and the title of each paper is included. The authors have contributed extensively from their own original work, some of which has not been previously published.

The purely chemical aspects of the subject matter are taken in conventional sequence. An introduction on historical lines is followed by a chapter on the preparation of the dithiocarbamates; their properties are discussed from a physico-chemical viewpoint and a chapter on their chemical reactions introduces a number of important derivatives, such as the thiuram sulphides and the isothiocyanates.

The heading "Analysis of dithiocarbamates and thiuram sulphides" is the somewhat misleading title of the chapter on the determination of these substances in mixtures. The assay of the "pure" dialkyldithiocarbamates by direct titration with standard iodine is not mentioned. Six pages are devoted to a discussion of the uses of dithiocarbamates in inorganic analysis. Twelve differently substituted dithiocarbamates have found use in the determination of twenty-six metals, and a useful Table provides literature references for the analytical methods that have been employed for these purposes.

The rest of the book, comprising nearly half its bulk, deals with the biological reactions and applications of these substances. Individual chapters cover biochemical investigations, applications in clinical medicine, fungicidal action and agricultural applications. The authors, who are with the Canada Department of Agriculture, have a particular interest in the last two subjects. The important applications of these sulphur compounds in the rubber industry are not considered, although it is made clear that much of the purely chemical information made available originated from research related to rubber technology.

Painstaking authorship and excellent book production have gone hand in hand to produce a volume that will prove invaluable to all who have an interest in this class of compounds.

W. C JOHNSON

LABORATORY ORGANIZATION AND ADMINISTRATION. By K. GUY, F.I.S.T. Pp. xiv + 386. London: Macmillan & Co. Ltd.; New York: St. Martin's Press. 1962. Price 50s.

The author of this book is the Chief Laboratory Technician in the Department of Chemistry and Chemical Engineering in the University of Natal. He received his early training in London and at one time was Chief Technician in the Chemistry Department of the Sir John Cass College.

With this background therefore it is not surprising that he has written a useful and extremely practical book on the management, design and maintenance of laboratories, and in particular, of chemical laboratories.

He writes, in a common sense way, about the designing of laboratories and about the precautions that must be taken in the installation of laboratory equipment, *e.g.*, balances. His chapter on stores management could be read with advantage by laboratory stewards in many industrial, university, college and school laboratories.

In 70 pages of text the author provides the reader with a great deal of information on safety precautions in laboratories, including, *e.g.*, protective measures against radiation. It is obvious that this chapter has been written from the point of view of one who has spent a great deal of his working life "near the bench" and with the right philosophy.

Projection methods useful in the lecture theatre are described and there is a great deal of sound information on the filing of laboratory records and technical information. Some attention is also paid to the organisation of exhibitions and demonstrations.

There is no doubt that this book will be welcome in many chemical laboratories whether industrial or academic. Our approval, however, must be qualified. In a general book of this kind, a great deal of the information on such specialised topics as biological reagents should have been omitted; the descriptions of chemical reagents and solutions could then have been extended with great profit.

Finally, and one says this with a certain amount of regret, there are parts of the book that contain rather too many sentences with subjects in the singular and verbs in the plural. Incidentally, on page 147, the volume of sea water should be given.

J. HASLAM

KOMPLEXONE IN DER CHEMISCHEN ANALYSE. By RUDOLF PŘIBIL. Pp. xxii + 474. Berlin: Veb Deutscher Verlag der Wissenschaften. 1961. Price DM 56.

There can be no doubt that Dr. Přibil is, more than most others, responsible for the present high status in inorganic analysis of complexometric titration with ethylenediaminetetra-acetic acid. Those who have been privileged to hear and see his elegant lecture/demonstrations on his all too infrequent visits to the English-speaking countries will be familiar with his mastery of the topic, and certainly the author's name is known and at least some of his procedures and indicators are used in every laboratory where the EDTA bottle is on the shelf.

The Czech text of this book was first published in 1953 and the second edition appeared in 1956. The latter has now been translated into German, and a small appendix, dated 1960, has been added that cites some new indicators produced in the author's own laboratories or those of his associates, and a few applications to gravimetry, colorimetry, polarography, etc.

The text is introduced by a review of the nature of complexes and then proceeds to examine the basic aspects of the reactions between complexones and metal-ions in aqueous solution. The main part of the text on analytical applications comes next, with separate chapters on the masking action of EDTA in gravimetry, titrimetry (non-complexometric), colorimetry, polarography, chromatography, electrophoresis and qualitative inorganic analysis. A short chapter deals with the determination of EDTA itself, and the last two chapters, which occupy about half of the text, deal with direct titrimetric applications of EDTA. The first of these giant chapters discusses the determination of metals by EDTA under the heading of the complexometric indicator used, *e.g.*, murexide for copper, nickel, cobalt, calcium, etc. It also takes into account physico-chemical methods of end-point detection, and considers masking agents. The final chapter deals with practical applications of complexometric methods to the determination of selected metal ions in technical materials such as alloys, electroplating baths, pharmaceuticals, plant substances and paper. Lastly, there is a short appendix added to the 1956 Czech edition and the slightly larger appendix mentioned above.

This brief review of the subject matter of the text rather conceals the extensive treatment afforded to each topic and even the scope of the book. Beyond doubt, this book by Přibil is the most detailed and well-informed text-book on this topic, less encyclopaedic than some perhaps, but only just so, and full of skilful assessment of the techniques and procedures. It is a most outstanding book and should make a great impact.

The chronology of this text has already been cited and in this, to my mind, lies its only fault. Thus, though it was published in 1961, up to p. 440 of the text, there are practically no references beyond 1955, and the author's appendix gives a rather incomplete coverage up to 1958. This, however, is no reflection on the book, which paints an accurate picture of the present situation in EDTA titrimetry, with only some of the more recent advances, in which new complexones and more specific indicators, etc., are used, not present. However, this eventuality is bound to be found in any book dealing with a topic that expands still with explosion-like rapidity. It is to be hoped that any further translation into a world-wide language of a new edition will not have to wait so long in future.

For all who are concerned with complexometric analysis this book must be regarded as absolutely indispensable. Přibil in print in indisputably the equal of Přibil at the laboratory bench.

T. S. WEST

METHODS OF ORGANIC ELEMENTAL MICROANALYSIS. By G. INGRAM, A.R.I.C. Pp. xvi + 511. London: Chapman & Hall Ltd. 1962. Price 75s.

There are many who would argue that microchemistry has no valid claim to be regarded as a subject in its own right; certainly the term has defied any agreed definition, for the boundaries of the subject may be set as wide, or as narrow, as the individual requires. But the wider the limits are set the greater the controversy is likely to rage; there were, for example, subjects discussed at Birmingham in 1958 that some were surprised to find classified as microchemistry. With the contents of Ingram's "Organic Elemental Microanalysis," however, there can surely be little dispute. Nor can it be denied that many of the methods the author describes represent a technique so specialised that there is full justification to classify them as microanalysis, separate and distinct from the routine working of the general analyst.

Having made a case for regarding microanalysis as a specialist branch of analytical chemistry let it be said that in few other divisions of the analyst's work is there quite so much need for reliance on the manipulative skill and craftsmanship of the individual. In Part I of his book, which deals with the determinations of carbon, hydrogen, oxygen, nitrogen, sulphur and the halogens, the author reveals himself as the complete master of his craft. The selected methods that he gives, in several instances inspired by his own researches, are described with the scrupulous attention to detail that is so helpful to the newcomer to this field of work. Not all established practitioners will accept every point the author makes, for each laboratory develops its own traditions and preferences and, indeed, its own myths. The defence of the use of a selenium - mercury catalyst in Kjeldahl digestions will find its opponents; so will the use of a boric acid solution in ammonia distillations. Sometimes, too, the author has had to rely on the literature without, one would imagine, the benefit of having tested the method for himself, for example, McCutchan and Roth's procedure for decomposition of nitro-compounds with thiosalicylic acid. This is understandable, however, for with so large a field to cover no analyst could be expected to have tried every variant in the extensive literature. What is clear, however, is that those methods that the author gives in detail are distilled from his many years of practical experience.

Part 2 of the book deals with the determination of metals and non-metals and Part 3 with determinations on the microgram scale. It will be noted that no attempt has been made to describe the determination of functional groups; in his decision to confine himself to the determination of the elements the author has, one feels, made a wise decision. The size of just over 500 pages is a convenient one, well-suited to the laboratory handbook that this is intended to be and that it will surely become.

The book is well produced in a pleasing modern style, although some confusion may arise from the weight given to various sub-headings within a chapter—this seems particularly to be so in Chapter 4 on the Determination of Halogens. But this in no way affects the general conclusion if you are concerned with organic elemental microanalysis, buy this book. The consistently high quality of the many diagrams is alone worth the money. C. A. JOHNSON

ABSORPTIONSSPEKTROSCOPIE IM ULTRAVIOLETTEN UND SICHTBAREN SPEKTRALBEREICH. By BRUNO HAMPEL. Pp. vi + 109. Braunschweig, Germany: Friedr. Vieweg & Sohn. 1962. Price DM. 16.80.

After 16 pages of introductory matter the author briefly discusses photographic methods, because, as he says, they are becoming obsolete. He then describes photo-electric spectrophotometry in general terms and three commercial instruments (Zeiss PMQ II, Unicam SP500, Beckman DK2) in more detail. This is followed by an account of technical methods and procedures. Finally, there is a full chapter on applications of absorption spectroscopy, covering the relationships between constitution and light absorption, chromophoric and auxochromic groups, some examples of constitutional problems elucidated by spectroscopy and some quantitative analytical problems.

This is an orthodox presentation, and for its size the book covers a lot of ground very well. It is presumably intended to provide an introduction to the subject that German-speaking students can afford to buy. The book is well printed, and the strong paper cover should give it a reasonable life. R. A. MORTON

STRAHLUNGSMESSUNG IM OPTISCHEN SPEKTRALBEREICH: MESSUNG ELEKTROMAGNETISCHER STRAHLUNG VOM ULTRAVIOLETT BIS ZUM ULTRAROT By GEORG BAUER. Pp. viii + 181. Braunschweig, Germany: Friedr. Vieweg & Sohn. 1962. Price DM 16.80.

This is Volume 16 in a series entitled "*Verfahrens- und Messkunde der Naturwissenschaft.*" It contains a good deal of physical optics and of fundamental theory. There is a good account of light sources of various kinds currently in use. Methods of dispersing radiation are described, and chapters on thermal and photo-electric detectors follow. The use of monochromators and of light filters is described, and the sensitivity of various detecting devices is discussed in detail.

This is a paper-back volume, well printed and illustrated and reasonably priced for the nature of its contents. It is scholarly, thorough and up to date wherever I have been able to test it.

R. A. MORTON

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Reprinted from Pure and Applied Chemisty, Vol. 5, Nos. 3-4.

DÜNNSCHICHT-CHROMATOGRAPHIE: EIN LABORATORIUMSHANDBUCH. By H. R. BOLLIGER, M. BRENNER, H. GÄNSHIRT, H. K. MANGOLD, H. SEILER, EGON STAHL and D. WALDI. Edited by EGON STAHL. Pp. xvi + 534. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 56.

FEBRUARY, 1963 ol. 88, No. 1043

THE ANALYST

EDITORIAL

WHEN, in 1924, Council appointed the Standing Committee on Uniformity of Analytical Methods to co-ordinate investigations into methods of analysis, it took—without knowing it—the first step towards the production of a book that the Society is publishing in a few days' time.* For the Standing Committee was the direct forerunner of the Analytical Methods Committee, and from the careful collaborative investigations of their various Sub-Committees has come the collection of tried and tested methods that form the first part of "Official, Standardised and Recommended Methods of Analysis."

The first of the Sub-Committee's reports appeared in *The Analyst* in 1927, and it was soon clear that existing methods had to be re-defined and revised, and that often new ones had to be produced, if they were to give results of acceptable precision in the hands of several analysts working in different laboratories. The high regard accorded to the methods resulting from these investigations is reflected in the large proportion that have subsequently been adopted unchanged, or with only minor amendments, as standard methods by such official bodies as the British Standards Institution, the British Pharmacopoeia Commission and the Pharmaceutical Society of Great Britain. Even so, the Society is continually extending the scope of these investigations, both through its own Analytical Methods Committee and in active co-operation with government departments, trade associations and other learned societies. These joint Committees also have recommended methods of analysis which have been published from time to time in *The Analyst.* One large group of methods, on Trade Effluents, was subsequently re-issued as a book.

Both the great popularity of the Trade Effluents book, and the increasing difficulty of referring to methods scattered over 35 volumes of *The Analyst,* convinced the Committee that a collected edition of the methods would be of considerable value to analysts. This collection forms the first part, extending over 340 pages, of the new publication. The Society acknowledges its debt to the Association of British Chemical Manufacturers, the Association of British Manufacturers of Agricultural Chemicals, the Inter-Departmental Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage and the Pharmaceutical Society of Great Britain for agreeing to the inclusion of methods developed by various joint committees.

But these methods cover no more than a part of the field of analysis. The exhaustive testing of procedures takes time, even when that time is so freely given as it has been by the many members of the score and more Sub-Committees and Panels engaged on the investigations. So, shortly after the second World War, a Standard Methods Committee was formed to collect, through a group of specialists, recommendations of reliable published methods for a variety of tests covering a wide range of materials. These compilations, in the form of classified lists of references, were published in 1951 as the Society's "Bibliography of Standard, Tentative and Recommended or Recognised Methods of Analysis," a book that for some time now has been unobtainable. The second part of the new publication comprises a completely revised and considerably augmented new edition of this Bibliography. Besides the British and American authorities quoted in the first edition, it includes references to some French and German sources, and to some in other languages for which translations may be readily available.

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* "Official, Standardised and Recommended Methods of Analysis," Compiled and Edited for the Analytical Methods Committee of the Society for Analytical Chemistry by S. C. Jolly, B.Pharm., B.Sc., A.R.I.C., M.P.S. Cambridge: W. Heffer & Sons Ltd. *for* the Society for Analytical Chemistry. Price £6 6s.

In this one publication an analyst can find details of procedures on which he can rely, even though he may never have met the particular analytical problems before. The bibliographical section gives him references to equally reliable methods that are "official" or have been "standardised," and also gives him a great deal of the guidance in the sphere of "recommended" methods that he could expect from an experienced senior colleague. He will undoubtedly come to share the Society's gratitude to the members of the investigating Sub-Committees, the compilers of the Bibliographical sections and the book's editor for their considerable efforts in making this book a reality.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, February 6th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. A. J. Amos, O.B.E., B.Sc., F.R.I.C.

The subject of the meeting was "Particle Size Analysis" and the following papers were presented and discussed: "The Size Analysis of Insoluble Drugs," by M. J. Thornton, B.Sc., A.R.I.C.; "Particle Size Analysis in the Formulation of Pesticides," by C. G. L. Furmidge, B.Sc., Ph.D., A.R.I.C.; "Particle Sizing in Aerosol Systems," by D. A. Blyth, B.Sc., J. M. Creasey and N. W. Wootten, B.Sc., A.Inst.P.

MIDLANDS SECTION AND MICROCHEMISTRY GROUP

A JOINT Meeting of the Midlands Section and the Microchemistry Group was held at 6.30 p.m. on Friday, December 14th, 1962, in the Chemistry Theatre, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Vice-Chairman of the Midlands Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "The Determination of Carbon and Hydrogen in Organic Materials." by Miss A. M. G. Macdonald, M.Sc., Ph.D., A.R.I.C.

The meeting was preceded by a tour of the new laboratories in the Haworth block of the University.

MIDLANDS SECTION

A JOINT Meeting of the Section with the Birmingham and Midlands Section of the Royal Institute of Chemistry was held at 7 p.m. on Thursday, January 24th, 1963, at the Technical College, Stoke-on-Trent. The Chair was taken by the Vice-Chairman of the Midlands Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Recent Advances in Polarography," by G. F. Reynolds, M.Sc., Ph.D., M.R.S.H., F.R.I.C.

MICROCHEMISTRY GROUP

THE thirty-seventh London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, December 19th, 1962, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

The meeting took the form of a Review Meeting, at which any subject related to those already covered in the Discussion Meetings was raised.

THE thirty-eighth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, January 23rd, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

A Discussion on "The Microdetermination of Mercury" was opened by R. F. Milton, B.Sc., Ph.D., F.R.I.C., M.I.Biol.

BIOLOGICAL METHODS GROUP

THE eighteenth Annual General Meeting of the Group was held at 6.30 p.m. on Thursday, December 13th, 1962, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. J. S. Simpson, F.I.M.L.T. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. W. A. Broom. *Vice-Chairman*—Dr. M. W. Parkes. *Hon. Secretary and Treasurer*—Mr. K. L. Smith, Standards Department, Boots Pure Drug Co. Ltd., Nottingham. *Members of Committee*—Dr. Jillian M. Bond, Mr. D. C. M. Adamson, Mr. L. C. Dutton, Dr. F. W. Norris, Mr. J. S. Simpson and Mr. G. Sykes. *Hon. Recorder*—Miss A. M. Jones. Mr. D. M. Freeland and Dr. J. H. Hamence were re-appointed Hon. Auditors.

Immediately following the Annual General Meeting a Discussion Meeting on "The Assessment of Vitamins in Feeding Stuffs" was opened by A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C.

ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL

THE Inaugural Meeting of the Atomic Absorption Spectroscopy Discussion Panel of the Physical Methods Group was held at 6.30 p.m. on Wednesday, December 12th, 1962, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The Chair was taken by the Chairman of the Panel, Mr. W. T. Elwell, F.R.I.C.

The following paper was presented and discussed: "Aspects of Atomic Absorption Analysis," by D. J. David, M.Sc. (see summary below).

ASPECTS OF ATOMIC ABSORPTION ANALYSIS

MR. D. J. DAVID said that, since the introduction of atomic absorption analysis and the apparatus essential for it by Walsh in 1953, research in Australia and New Zealand had been conducted along three main lines. These were—

(*a*) The study of vaporisation of samples in flames, entailing examination of inter-element effects arising from variations in general composition of samples, interaction between flame gases and sample and the effect of organic solvents.

(*b*) The study of alternative means of vaporisation of samples, notably use of the sputtering chamber for metallic samples.

(*c*) The search for means of increasing sensitivity by choice of absorbing line and modification of apparatus.

Recent research in the United States had dealt mainly with the study of increase in length of light path through the vaporised sample as a means of gaining sensitivity and the use of a continuum as source of radiation in place of line sources. The speaker discussed these points and emphasised those upon which further advances might depend.

In the discussion that followed, many members of the audience expressed opinions on these points and on other matters of technique that held a general interest.

The Spectrophotometric Determination of Sub-microgram Amounts of Fluorine in Biological Specimens

By R. J. HALL*

(*Agricultural Research Council Institute of Animal Physiology, (Pharmacology Unit), Babraham, Cambridge*)

A method is described for the accurate determination of sub-microgram amounts of fluorine involving the new direct colour reaction with alizarin complexan. The fluoro-chelate formed with lanthanum alizarin complexanate is extracted into isobutanol containing hydroxylamine hydrochloride. Fluorine is collected from ashed specimens on to magnesium succinate treated filter-paper by diffusion, in small polythene bottles, induced by 50 per cent. perchloric acid containing silver sulphate. Recoveries for 0·1 to 1·0 μg of fluorine are over 95 per cent. Problems associated with determining these small amounts are discussed. The method lends itself well to determining fluorine in water and in biological materials such as blood and urine.

THE detection of fluoride with alizarin complexan (3-[di-(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone) was first reported by Belcher, Leonard and West.[1,2] This new reagent differs from others in that its use appears to give the first direct colour reaction for the fluoride ion. Leonard and West[3] later noted that the blue cerium fluoro-chelate of alizarin complexan was soluble in pentanol containing n-tributylamine. Their observation prompted a further study of the conditions for forming the cerium and lanthanum fluoro-complexes, and especially their solubilities in organic solvents containing amines. The use of hydroxylamine hydrochloride in isobutanol as a solvent for the lanthanum fluoro-chelate has made possible the development of a method for determining fluoride in the sub-microgram range. By modifying a diffusion technique for the collection of hydrofluoric acid described in an earlier paper,[4] reproducible and quantitative recoveries of 0·1 to 1·0 μg of fluorine have been obtained; the method described below is proposed for the determination of such levels in biological specimens.

METHOD

REAGENTS—

Reagents should be of analytical grade when possible.

Magnesium succinate, 0·2 M—Dissolve 5·13 g of fluoride-free magnesium succinate, $Mg(C_4H_4O_4)_2$, in water, and dilute to 100 ml; store in a polythene bottle at 2° C.

Silver sulphate in perchloric acid—Approximately 0·25 M silver sulphate in 70 per cent. w/v perchloric acid. Mix 4 g of finely powdered silver sulphate with 2 ml of water and 23 ml of 72 per cent. perchloric acid in a 250-ml Erlenmeyer flask, and heat to about 80° C to dissolve the silver sulphate. Add a further 75 ml of 72 per cent. perchloric acid, and cool the solution.

Succinate buffer solution, pH 4·6 (0·04 M)—Dissolve 0·4724 g of succinic acid in 96 ml of water (with warming to aid solution). Cool the solution, add 3·2 ml of N sodium hydroxide, and make up to 100 ml with water. The pH should be checked potentiometrically. Best results are obtained when the solution is fresh, but, if necessary, it can be stored at 2° C in a polythene bottle and prepared weekly.

Alizarin complexan solution, 0·0005 M—Suspend 38·5 mg of reprecipitated alizarin complexan in 2 ml of water in a test-tube; add 0·2 ml of M sodium acetate, and warm the mixture to dissolve the complexan. Transfer the solution to a 200-ml calibrated flask, and make up to the mark with water. Store at 2° C in a dark bottle. The solution is stable for a considerable length of time.

To reprecipitate the alizarin complexan (obtainable from Hopkin and Williams Ltd.) dissolve 1 g in about 10 ml of 0·5 M sodium acetate, and heat to about 80° C; if necessary, add more sodium acetate solution until the solid has dissolved. Transfer the solution to a stoppered 100-ml measuring cylinder, and dilute to 100 ml with water. Carefully add hydrochloric acid dropwise until a heavy orange precipitate forms. The precipitation is continued for some hours at 0° to 2° C. Remove the supernatant liquid, separate the precipitate from residual liquid by spinning in a centrifuge, wash twice with 20 ml of ice-cold water, and dry at 37° C.

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Lanthanum nitrate, 0·001 M—Dissolve 43·3 mg of lanthanum nitrate, $La(NO_3)_3.6H_2O$, in water to make 100 ml of solution.

Buffered lanthanum alizarin complexanate—By pipette, place 30 ml of the 0·0005 M alizarin complexan, with constant mixing, into 50 ml of the succinate buffer solution and 20 ml of lanthanum nitrate solution. Then add 25 ml of t-butanol to the mixture. Prepare freshly each day.

Hydroxylamine hydrochloride solution—Prepare a molar solution by dissolving 6·95 g of hydroxylamine hydrochloride (recrystallised from 75 per cent. v/v ethanol at 2° C, and dried at 60° C) in water to make 100 ml of solution.

Extracting solvent—Shake 3 ml of the hydroxylamine hydrochloride solution with 97 ml of isobutanol until the two liquids have become homogeneous. The solvent will keep for several days, but a slight precipitate tends to form after a time.

Stock fluoride solution, 100 *μg of F^- per ml*—Dissolve 221·05 mg of pure sodium fluoride in water, and dilute to 1000 ml.

Standard fluoride solutions, 10 *μg of F^- and* 2 *μg of F^- per ml*—Dilute a 10-ml and a 2-ml portion of the stock fluoride solution to 100 ml. These solutions should be freshly prepared each week.

All fluoride solutions should be stored in screw-capped polythene bottles.

Magnesium succinate solution, fluoride-free—First prepare a 5 per cent. w/v solution of succinic acid in water distilled in an all-glass apparatus. Wash 3 g of magnesium turnings with 100 ml of distilled water in a 500-ml Erlenmeyer flask. Discard the washings, and replace by 100 ml of fresh distilled water to which 50 ml of the 5 per cent. succinic acid have been added. After about one minute, carefully pour off the acid, and wash the magnesium twice with 100 ml of water distilled in an all-glass apparatus. Suspend the magnesium in 100 ml of water distilled in an all-glass apparatus and 100 ml of 5 per cent. succinic acid. When the main reaction has subsided, evaporate the contents of the flask to about 80 ml and then filter through a washed Whatman No. 42 filter-paper or spin in a centrifuge (radius about 4 inches) at 4000 r.p.m. in cellulose nitrate or polythene tubes. Evaporate to dryness at 100° C overnight, and then grind the white residue to a powder with a glass pestle and mortar. Prepare a 0·2 M solution of the magnesium succinate.

Lithium hydroxide solution, fluoride-free—Dry with filter-paper lumps of lithium metal that have been stored in paraffin spirit. Remove the outer crust with a sharp knife or tin shears, and quickly weigh about 4 g of the clean metal. Cut into small pieces the size of a pea, and drop into 500 ml of water distilled in all-glass apparatus contained in a 1000-ml Erlenmeyer flask. When all the lithium has dissolved, check the normality of the solution by titration against potassium hydrogen phthalate, and then adjust the concentration to 1 N.

PREPARATION OF SAMPLE—

Samples of 1 ml of blood, cerebrospinal fluid, urine, etc., are measured into small platinum crucibles, mixed with 0·3 ml of N lithium hydroxide and 0·2 ml of 0·2 M magnesium succinate and dried at 110° C. Bone, vegetable and soft animal tissues are dried at 110° C for several hours, finely ground, and 0·2 g is mixed with alkali and magnesium succinate as above. The crucibles are covered with lids and placed in a small metal canister having a tightly fitting lid; the canister is then placed in a cold muffle furnace, and the temperature is slowly raised to 400° C and maintained for about 15 hours, usually overnight. The canister is removed from the furnace while still hot. The bone ash is carefully broken up with a small nickel spatula and transferred to a 100-ml calibrated flask; the remaining traces of ash are treated with small portions of 0·5 N perchloric acid to a total of 5 ml, and the dish is washed with about 10 ml of water, all the washings being added to the ash in the flask. One millilitre of 72 per cent. v/v perchloric acid is added, and the flask is gently warmed to about 60° C, when the ash will be completely dissolved apart from a few particles of carbon; the volume in the flask is then adjusted to the mark with water. Samples of 1 ml are taken for the collection of hydrofluoric acid in the diffusion bottles. The ash of blood, cerebrospinal fluid, urine or tissue is similarly broken up and carefully transferred to a polythene diffusion bottle, the platinum dish is washed with 0·4 ml of 0·5 N perchloric acid and then twice with 0·3 ml, and the washings are also placed in the diffusion bottle.

Appropriate controls consisting only of the reagents are similarly prepared, as well as standards containing 0·1, 0·2, 0·5, 0·7 and 1·0 µg of fluoride in 1-ml volumes; when possible all diffusions should be carried out at least in duplicate.

DIFFUSION OF HYDROFLUORIC ACID—

The procedure already reported[4] for collecting fluorine by diffusion as hydrofluoric acid was modified; the changes made were as described below.

Rectangles of Whatman No. 541 filter-paper, 3·0 cm × 1·2 cm, previously extracted with hot distilled water, rinsed and dried, are used. These are pushed into the lumen of an adaptor of 5-mm internal diameter polythene tubing inside the well of the bottle cap, thus dispensing with the short length of glass rod originally used. The papers remain reasonably tightly held. The free 2 cm of the paper is impregnated with 15 µl of 0·2 M magnesium succinate placed 0·5 cm from the free edge. A graduated 0·1-ml pipette is useful for this purpose. The sample in a volume of 1 ml is placed in the bottle from a pipette; 2 ml of silver sulphate solution in 70 per cent. w/v perchloric acid is then immediately added from a burette, care being taken not to allow any acid to remain on the inside of the bottle neck. The cap with its filter-paper is screwed tightly into position. The screw threads of the polythene bottle caps and necks tend to wear and cause loss of hydrofluoric acid. This source of error has been overcome by sealing the junction between the cap and the bottle neck with hot wax. A suitable wax is a mixture of equal weights of ceresine and carnauba wax (melting point about 85° C), which is easily applied with a dropping pipette; 1 cm of the end of its stem is bent at an angle of 135° and fitted with a 2-ml rubber bulb. The bottle is tilted on its side at an angle of about 30°, and the wax, which should be very hot (so that it is vaporising), is allowed to run round the junction between the cap and bottle neck. Diffusion is continued for 24 hours at 60° C.

DETERMINATION OF FLUORIDE—

The papers are removed from the diffusion bottles with forceps and placed in stoppered 5-ml tubes. Lanthanum alizarin complexanate reagent (2·0 ml) is placed by pipette in the tubes, which are then put into a water bath at 60° C for 10 minutes. The colour of the alizarin reagent changes from magenta to blue, according to the amount of fluoride present. The tubes are cooled to room temperature, and the blue complex is extracted by shaking vigorously for 15 seconds each time with a 1·5-ml and then two successive 1-ml portions of the isobutanol solvent. The tubes are spun in a centrifuge (radius about 4 inches) for a few moments at about 2000 r.p.m. after each extraction to separate the solvent layer, and this is removed to another stoppered 5-ml graduated tube; a 2-ml graduated pipette having a finely pointed tip and fitted with a 2-ml rubber bulb is used for the transfer. To obviate the need for a separate pipette for each extract and to prevent contamination, the pipette is filled after each use to the 1-ml mark with the extracting solvent, and the washings are transferred to the extraction tube. In this way contamination is negligible.

After the volumes have been adjusted to 4·3 ml with solvent, the extracts are shaken for 30 seconds with 1 ml of water and then cooled to 0° C or lower. The aqueous phase is separated by spinning in a centrifuge for a short time, and, when necessary, the volume of the extract is adjusted to 4 ml. The extracts are then transferred to glass cells, and their optical densities are measured at 570 mµ or photometrically with a red filter.

Note that the diffusion bottles should be emptied under running cold water, washed out several times with distilled water only, and dried at 60° C. All tubes should be cleaned by soaking for a few minutes in dilute hydrochloric acid and then washing several times with distilled water. Platinum crucibles should be soaked in 10 per cent. perchloric acid, washed with distilled water, and heated in a bunsen flame until red hot. This procedure ensures that any traces of fluoride are volatilised.

EXPERIMENTAL RESULTS

All experimental observations were carried out in duplicate or triplicate, and a Unicam SP500 spectrophotometer was used for measuring optical densities.

SOLVENT FOR FLUORIDE CHELATE—

During the preliminary experiments it became evident that, although the blue complex obtained by the reaction of fluoride with the cerium or lanthanum chelate of alizarin complexan was easily extracted into n-pentanol containing tributylamine, the unreacted complexanate was also fairly soluble and there was a direct correlation between ease of extraction and concentration of the amine. Lanthanum alizarin complexanate alone when extracted into 0·08 per cent. v/v tributylamine in n-pentanol gave 30 per cent. of the optical density of the blue complex produced by 1 µg of fluoride in a standard volume of 4 ml. Many amines dissolved in alcohols will extract the fluoride complex with different efficiencies; these include methylamine hydrochloride and ethylamines, as well as tribenzylamine.[5] Hydroxylamine hydrochloride in isobutanol was by far the most satisfactory solvent because of the high solubility of the fluoro-chelate and low solubility of the lanthanum complexanate. Blank values were reduced to an average of 4 per cent. of the optical density of the complex obtained with 1 µg of fluoride in a volume of 4 ml.

EFFECT OF BUFFER AND pH ON FORMATION OF FLUORIDE COMPLEX WITH LANTHANUM ALIZARIN CHELATE—

All work so far reported on the reaction of fluoride with the cerium or lanthanum chelates of alizarin complexan has involved the use of acetate buffer solutions ranging from pH 4·3 to 5·1. In the work described here it was confirmed that pH 5·0 was optimal for an acetate-buffered reagent,[6] but it was also noticed that other buffer systems of the same pH range profoundly affected the reaction. Thus boric acid buffered reagent (final concentration 0·1 M) produced a more intense blue fluoro-chelate than did acetate, but a succinate-buffered reagent, pH 4·6 (final concentration 0·02 M), also markedly increased the speed of reaction to give greater sensitivity and lower blank values. It is suggested that the solubility of the buffer systems in the organic solvent may be a factor governing the efficiency of extraction. Fluoro-chelates produced with borate- or succinate-buffered reagents were much more easily extracted than were those produced with acetate-buffered solutions; acetate buffers are appreciably soluble in the alcohol solvents and presumably inhibit the effect of the amine. Fig. 1 and Table I show a striking comparison of optical densities of the extracts of fluoro-chelate produced in acetate- and succinate-buffered alizarin complexan reagents. The peak at pH 4·6 in the succinate range appears to be characteristic, irrespective of the method of extraction.

TABLE I

OPTICAL DENSITIES AT 570 mµ PRODUCED BY 1 µg OF FLUORIDE

Reaction carried out with lanthanum alizarin complexanate reagents prepared with acetate and succinate buffers, and extracted into 4 ml of solvent. Optical densities corrected for blank value

pH	Optical density Acetate (final concentration 0·1 M)*	Succinate (final concentration 0·02 M)†
4·0	0·090	0·159
4·2	0·099	0·212
4·4	0·119	0·234
4·6	0·119	0·264
4·7	0·120	0·257
4·8	0·122	0·249
5·0	0·123	0·253
5·2	0·110	0·259

* Extracted with 0·08 per cent. v/v of tributylamine in n-pentanol.

† Extracted with 0·03 M hydroxylamine hydrochloride in iso-butanol.

EFFECT OF TEMPERATURE AND TIME ON REACTION BETWEEN FLUORIDE AND LANTHANUM ALIZARIN COMPLEXANATE—

Results of the influence of temperature and period of reaction on the formation of the fluoro-chelate are shown in Table II. Fluoride (1 µg) in 2 ml of the alizarin complexan reagent was taken. Prolonged immersion at 100° C caused fading of the blue fluoro-chelate.

It was decided to adopt 10 minutes at 60° C as the standard procedure.

TABLE II

EFFECT OF TEMPERATURE AND TIME ON REACTION BETWEEN FLUORIDE AND LANTHANUM ALIZARIN COMPLEXANATE

Temperature, °C	Time, minutes	Optical density (4-ml extract)
20	5	0·245
20	10	0·244
20	30	0·245
20	45	0·246
20	60	0·251
60	10	0·266
100	5	0·248

COMPARISON OF OPTICAL DENSITY OF DIRECT REACTION MIXTURES AND EXTRACTS—

Table III compares the optical densities of the lanthanum fluoro-chelate in succinate buffer solution at pH 4·6 read directly and in isobutanol extracts; the superiority of the extraction procedure is clearly indicated.

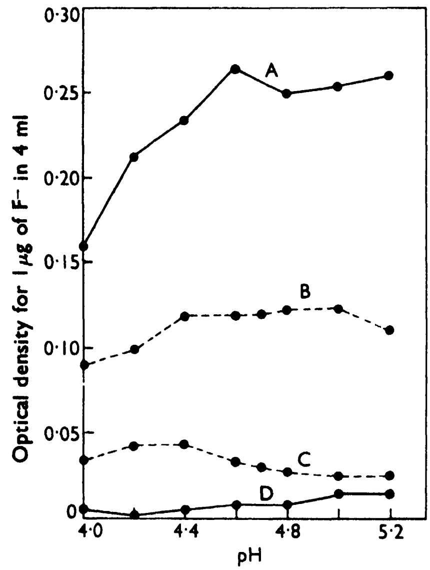

Fig. 1. Effect of pH on optical density of extracts of the fluoro-chelate of lanthanum alizarin complexanate prepared with acetate and succinate buffers: curve A, chelate with succinate buffer; curve B, chelate with acetate buffer; curve C, blank test with acetate buffer; curve D, blank test with succinate buffer

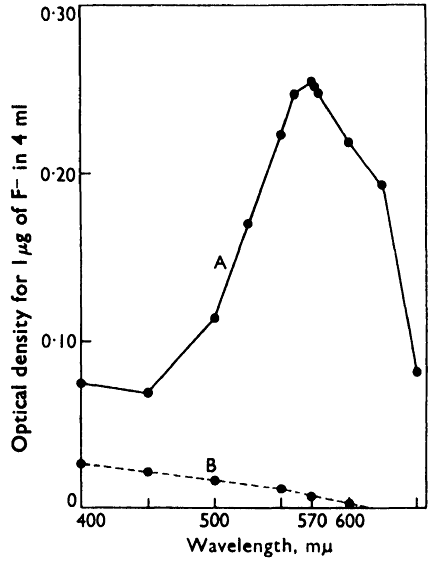

Fig. 2. Absorption curve of the fluoro-chelate of lanthanum alizarin complexanate prepared with succinate buffer at pH 4·6: curve A, chelate; curve B, blank test

MEASUREMENT OF COLOUR—

Fig. 2 shows the absorption curve of the fluoro-chelate in isobutanol - hydroxylamine hydrochloride obtained with the lanthanum-based reagent; that of the cerium reagent is similar. It can be seen that the optimum wavelength is 570 mμ. The general shape of the curve is similar to that obtained with the chelate in aqueous solution, the only difference being a slight shift of the curve to the left; the peak is at 615 mμ in aqueous medium.

RECOVERY TESTS—

Representative recoveries of fluorine from the diffusion bottles are shown in Table IV and indicate that virtually all the fluorine within the range is collected on the paper wicks under the conditions of the test.

TABLE III

COMPARISON OF OPTICAL DENSITIES OF REACTION MIXTURES AND THEIR ISOBUTANOL EXTRACTS

The volumes used were 4 ml throughout, and blank values were not subtracted

	Optical density	
Fluorine present, μg	Direct reading on reaction mixture at 615 mμ	Reading on extract at 570 mμ
0	0·112	0·008
0·1	0·114	0·034
0·2	0·128	0·060
0·4	0·155	0·111
0·6	0·180	0·163
0·8	0·205	0·214
1·0	0·248	0·265

The method for determining the recovery was to measure the colour obtained from the fluorine collected on the paper against that obtained with the same amount of fluorine added directly by pipette to 2 ml of the lanthanum reagent. A specimen of the filter-paper with 15 μl of the magnesium succinate solution was also placed in the reagent to provide a blank solution. The third decimal place shown in the figures is only approximate.

Recovery of fluorine added to blood and urine carried through the entire procedure has been confined to the 0·2- and 0·5-μg levels; recoveries were from 96 to 114 and from 95 to 102 per cent., respectively.

TABLE IV

RECOVERY OF FLUORINE FROM DIFFUSION BOTTLE

Amount of fluoride added, μg	Amount of fluoride found, μg	Recovery, %
0·1	0·112	112
	0·089	89
	0·092	92
	0·096	96
	0·091	91
0·2	0·200	100
	0·195	97
	0·193	96
	0·200	100
	0·188	94
0·5	0·487	97
	0·496	99
	0·479	96
	0·486	97
	0·500	100
1·0	1·000	100
	0·965	96·5
	1·010	101
	0·992	99
	1·008	101

INTERFERENCES—

Belcher and West[6] reported on substances interfering with the formation of the cerium fluoro-chelate of alizarin complexan. They found that several anions and cations profoundly influence the reaction. Some of these observations have been confirmed in this laboratory and equally apply to the lanthanum chelate. The formation of blue complexes in the succinate-buffered lanthanum alizarin complexanate reagent with small amounts of cobalt and nickel has been observed in the absence of fluoride. These complexes are to some extent also extractable into the isobutanol solvent. Surprisingly, however, the presence of 3 μg of nickel depresses the optical density of the lanthanum fluoro-chelate from 1 μg of fluoride in the organic solvent by 20 per cent., presumably by inhibiting the extraction. When the cobalt and nickel levels are increased to 30 and 10 μg, respectively, the production of the fluoro-chelate is almost completely inhibited. Interference of this nature would not be expected in the collection of hydrofluoric acid by diffusion. Although these particular observations were not pursued, further work may provide more information on the specificity of the reaction for fluoride.

DISCUSSION OF THE METHOD

The accurate determination of small amounts of fluorine has been a major analytical problem for many years, and the introduction of a direct colour reaction is an important advance in this field. Although the colour reaction of the fluoride ion with the lanthanum and cerium complexes of alizarin complexan appears specific, serious interference may be encountered from several metal ions and radicals,[6] and, although most may be masked and removal by extraction is necessary in only a few instances (chiefly coloured ions), such an additional operation will complicate the analysis. All interference is eliminated by the collection of the fluorine by diffusion on to magnesium succinate treated filter-paper. Magnesium succinate affects the colour reaction hardly at all, and recoveries from the diffusion bottles of 0·1 to 1·0 µg of fluorine are quantitative for all practical purposes. The inclusion of a silver salt with the concentrated perchloric acid immobilises any chloride ions; a step also adopted by Stegemann and Jung,[7] who collected the hydrofluoric acid on alkali-soaked poly(vinyl chloride) paper. A more recent application of diffusion methods to the determination of fluoride has been reported by Frere,[8] who used plastic Conway units for the collection of hydrofluoric acid and adopted the reaction with lanthanum alizarin complexanate for its subsequent determination.

Further studies of the formation of the fluoro-chelates of alizarin complexanates confirmed the findings of Greenhalgh and Riley[9] that the lanthanum complexanate gave a more intense colour with fluoride ion than did the cerium complexanate. The main advantage of the lanthanum complexanate, however, is that its fluoro-complex is more soluble in the organic phase than is that of cerium. Greenhalgh and Riley[9] also reported increased sensitivity by including 12 per cent. of acetone in their lanthanum reagent, and Belcher and West[6] extended these findings to include methanol, ethanol and dioxan. In this investigation it was found that a much wider range of solvents apparently enhanced the visual sensitivity of the reaction when present in the mixture at concentrations from 10 to 20 per cent. by volume. However, after these reaction products had been extracted with the isobutanol - hydroxylamine hydrochloride mixture the optical densities of the blue fluoro-chelate extracts with one exception were always below that of an extract from an entirely aqueous reagent. The exception was t-butanol, which did not affect the extract, and was included to prevent the formation of precipitates at the junction of the organic and aqueous phases during the extraction. In the earlier part of the work traces of the disodium salt of ethylenediaminetetra-acetic acid were employed to prevent these precipitates, but later t-butanol was found to be more effective; it also tended to stabilise the reagent. Increased sensitivity was claimed by Belcher and West[10] to be obtainable by reading at 281 mµ the direct reaction in aqueous solution between fluoride and the lanthanum chelate. Although the optical densities obtained with this procedure are much higher, so also is that of the unreacted lanthanum reagent. Increases in the optical density of the lanthanum fluoro-chelate when extracted into the organic solvent were similarly measured at 281 mµ, but even the almost colourless blank extract had so high a reading at this wavelength that the procedure was unsuitable for measuring the smallest amounts of fluoride, which requires the blank value to be as near zero as possible.

The extraction of the fluoro-complex of the lanthanum complexanate into isobutanol - hydroxylamine hydrochloride mixture proved to be the best means of measuring the complex formed from less than 0·1 µg of fluoride. Many combinations of organic solvents and amines were tried. Although tributylamine in n-pentanol, mentioned by Leonard and West,[3] easily extracts the fluoro-chelate, it also extracts much of the unreacted lanthanum complexanate. The same is true of tribenzylamine in pentanol - s-butanol (30 + 70) suggested by Johnson and Leonard.[5] For determining the smallest amounts of fluoride, even with the isobutanol - hydroxylamine hydrochloride solvent, the extract of the reaction mixture still contains sufficient lanthanum complexanate to give a relatively high optical density for the blank solution. Fortunately, almost all of this colour can be removed by washing the extract with 1 ml of water. The fluoro-chelate seems to be reasonably insoluble in water. The test extract is similarly washed, and hence the fluoro-chelate is isolated free from extraneous red colour with an almost colourless blank solution. The advantages of the extraction method are shown clearly in Table III; in the range of 0·1 to 1·0 μg of fluoride, Beer's law is well obeyed for the extracts of the fluoro-chelate, but erratic correlation between fluoride and optical density was obtained when the direct reaction was measured. These findings are different from those of previous workers who, however, measured the reaction with much larger amounts of fluoride. Some preliminary tests have indicated that the fluoro-chelate in the isobutanol solvent can be re-extracted into a small volume of dilute alkali or buffer in aqueous solution. This method of concentration may lend itself to the measurement of even smaller amounts of fluoride.

In applying the method to determining fluorine in biological specimens, some further observations were made. Experience with former conventional methods of ashing, distillation and titration for collecting and measuring small amounts of fluorine makes the analyst aware of the need for carefully controlling potential sources of contamination. The proposed method revealed contamination from fluoride that hitherto well accepted procedures did not show. By the proposed method relatively large amounts of fluorine were found in many common laboratory reagents, frequently several micrograms per millilitre of molar solution; this particularly applied to salts of calcium, lithium and magnesium. But the most serious contamination of all came from the muffle furnace. Several micrograms of fluoride may be washed from the inside of an empty platinum crucible after being in a muffle furnace for a few hours at 400° C, even if the crucible is covered with a lid. When alkali is present in the crucible this source of contamination becomes greater still and subject to considerable variation. The problem may be overcome by placing the crucibles covered with their lids inside another metal container with a well fitting, but not air-tight, lid. Screw-cap lids are not suitable. Attempts to use crucibles of other materials, such as aluminium, nickel, stainless steel or silica, were without success. Losses were experienced of the small amounts of fluoride placed in the crucibles, possibly by adsorption to the surface of the crucibles—this is a well recognised danger in ashing procedures.

In order to fix the fluoride, lithium hydroxide with magnesium succinate was found to be the most suitable agent, and clean ashes were obtained.

The method has been successfully used for determining the fluorine content of water and of various animal and plant tissues and body fluids; it is hoped that details will appear later in another publication. One important observation arising from the use of the proposed method concerns the fluoride levels of human and animal blood. Hitherto, most workers using conventional methods have reported figures of about 2 μg of fluoride per ml of whole blood. More recently, Singer and Armstrong[11] obtained values of 0·12 μg per ml of plasma or serum; they collected the fluorine by means of a special micro still, after ashing with magnesium oxide. Many determinations of fluoride in human, dog and sheep plasma and whole blood have now been carried out in this laboratory, and only rarely has any measurable amount been found in plasma and usually less than 0·1 μg per ml in whole blood. Some of the high figures reported in the past may be connected with the uncertainty of the thorium nitrate titration, which is well known to fluorine chemists.

There are many important problems still to be studied on the effects of fluorine on all forms of life, and it is hoped that this technique offers a practical approach to the accurate determination of extremely small amounts of this element and with materials normally available in most analytical laboratories.

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A Modified Zirconium-Alizarin Method for determining Fluoride in Natural Waters

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A modified zirconium - alizarin method, accurate to within 0·05 p.p.m. of fluoride, is described for determining fluoride in water without prior distillation. The difficulty of variation in colour was overcome by extracting the alizarin with pentanol. Interference from phosphate and other impurities has been overcome.

MOST methods for the colorimetric determination of fluoride are based on the fact that fluoride decreases the colour intensity of some metal complexes.[1,2,3,4,5,6] The zirconium - alizarin method[7,8,9] is probably the most satisfactory for determining fluoride in natural waters. However, it has the disadvantages that—

(*a*) the red and yellow colour mixture produced by the alizarin - zirconium reagent is different for each fluoride content;

(*b*) most of the impurities found in natural waters influence the results;

(*c*) the methods are accurate to only 0·1 p.p.m. of fluorine, even after distillation.

These disadvantages are overcome in the modification described here.

METHOD

REAGENTS—

Pentanol—Redistilled industrial pentanol is satisfactory. It must be clear and colourless.

Magnesium chloride solution, saturated, aqueous.

Sodium fluoride solution—A solution containing 0·1 mg of fluorine per ml.[10]

Zirconium oxychloride solution—Dissolve 0·177 g of zirconium oxychloride, $ZrOCl_2.8H_2O$, in about 300 ml of distilled water, add 17 ml of concentrated sulphuric acid and 50·5 ml of concentrated hydrochloric acid, cool, and make up to 500 ml with distilled water.[10]

Alizarin solution—Dissolve 0·3752 g of alizarin red S in 500 ml of distilled water.[10]

Alizarin - zirconium reagent solution—Mix equal portions of the alizarin and zirconium oxychloride solutions immediately before use.

Ferric chloride solution—Dissolve 1 g of ferric chloride, $FeCl_3.6H_2O$, in 50 ml of distilled water.

Sodium acetate solution, 25 per cent. w/v, aqueous.

PROCEDURE—

If the specific conductivity of the sample is below 500 μmho, add 1 ml of magnesium chloride solution per 500 ml of sample.

Use three glass-stoppered 100-ml cylinders (the total volume of each cylinder being at least 140 ml); in one cylinder (cylinder 1) place 100 ml of distilled water containing 1 ml of magnesium chloride solution per 500 ml and in the other two (cylinders 2 and 3) place 100-ml portions of sample. Add 1 ml of sodium fluoride solution to the contents of cylinder 3 and 1-ml portions of distilled water to the contents of cylinders 1 and 2.

Add 10 ml of alizarin - zirconium reagent solution to the contents of each cylinder, mix well, and allow the colour to develop for one hour in the dark. Then add to each 10 ml of pentanol, and shake well for one minute. Set aside in the dark until the top layer is clear (usually 15 minutes, but we allow 30 minutes).

Transfer with a dropper part of the pentanol layer from each cylinder to a separate 1-cm cuvette. Set a spectrophotometer at wavelength 430 mμ, adjust to read zero against the solution from cylinder 1, and then measure the optical densities of the solutions from cylinders 2 and 3.

CALCULATION—

If the optical density of the solution from cylinder 2 is E_2 and of that from cylinder 3 is E_3, then the concentration of fluorine in the water is given by the relation—

$$\frac{E_2}{E_3 - E_2} \text{ p.p.m.}$$

DISCUSSION OF THE METHOD

By extracting the yellow colour of the free alizarin with pentanol the interference caused by red zirconium lake was avoided, as was that caused by colour and slight turbidity of the water. Pentanol was chosen because alizarin sulphonic acid is soluble in water and alcohol. It was easily available and, as it is relatively insoluble in water, it was an obvious choice.

It was shown by adding different known amounts of fluoride to distilled and other waters and measuring the optical densities that the amount of free alizarin was directly proportional to the amount of fluoride in the sample (see Table I); waters of low conductivity were an exception. It was therefore necessary to increase the salt concentration of waters having low specific conductivity.

The optical density per 1 p.p.m. of fluorine decreases with increasing salt content in any particular water; this is most marked with aluminium salts (see Table I). Hence the necessity of using a third cylinder containing the sample to be tested, to measure the optical density per 1 p.p.m. of fluorine.

TABLE I

OPTICAL DENSITY PER 1 p.p.m. OF FLUORIDE FOR DIFFERENT WATERS

Sample	Fluoride present, p.p.m.	Optical density	Optical density per 1 p.p.m. of fluoride
1. Distilled water	0	0	—
	0·2	0·122	0·61
	0·5	0·255	0·51
	1·0	0·385	0·38
2. Distilled water containing 0·5 p.p.m. of aluminium*	0	0	—
	0·2	0·046	0·23
	0·5	0·144	0·29
	1·0	0·386	0·39
3. Artificial water†	0	0	—
	0·2	0·098	0·49
	0·5	0·140	0·48
	1·0	0·484	0·48
4. Distilled water containing 1 ml of magnesium chloride per 500 ml . .	0	0	—
	0·2	0·079	0·40
	0·5	0·212	0·42
	1·0	0·443	0·44
5. Buffelspruit water	0	0	—
	1·0	0·364	0·36
	2·0	0·702	0·35
	3·0	1·040	0·35
6. Artificial water† containing 0·5 p.p.m. of aluminium*	0	0	—
	0·2	0·067	0·34
	0·5	0·174	0·35
	1·0	0·359	0·36
7. Artificial water† containing 1 p.p.m. of aluminium* . .	0	0	—
	0·2	0·040	0·20
	0·5	0·106	0·21
	1·0	0·242	0·24
8. Distilled water containing 1 ml of magnesium chloride solution per 500 ml and 0·4 p.p.m. of phosphate	0	0	—
	0·2	0·078	0·39
	0·5	0·201	0·40
	1·0	0·411	0·41

* Added as $KAl(SO_4)_2.12H_2O$.

† Distilled water containing 125 p.p.m. each of calcium carbonate, magnesium sulphate and sodium hydrogen carbonate. The calcium carbonate was first dissolved in hydrochloric acid.

To show that the optical density of the blank solution was equal to that of the sample without fluoride, eight waters were made up and examined. The optical density of the distilled water blank was adjusted to 0·020.

The optical densities of waters 1 to 5 (see Table II) showed no significant differences, and the influence on the final result was less than 0·05 p.p.m. of fluorine.

TABLE II

INFLUENCE OF VARIOUS SUBSTANCES ON BLANK VALUE

Water No.	Composition	Optical density	Difference
1	Distilled water	0·020	—
2	Distilled water containing 0·5 p.p.m. of aluminium*	0·022	+0·002
3	Artificial water†	0·010	−0·010
4	Artificial water† containing 0·5 p.p.m. of aluminium*	0·018	−0·002
5	Distilled water containing 1 ml of magnesium chloride solution per 500 ml	0·015	−0·005
6	Distilled water containing 1 ml of magnesium chloride solution per 500 ml and 0·5 p.p.m. of phosphate	0·043	+0·023
7	Distilled water containing 1 ml of magnesium chloride solution per 500 ml and 1·0 p.p.m. of phosphate	0·063	+0·043
8	Distilled water containing 1 ml of magnesium chloride solution per 500 ml and 1·5 p.p.m. of phosphate	0·086	+0·066

* Added as $KAl(SO_4)_2.12H_2O$.

† For composition, see Table I.

TABLE III

ANALYSIS OF WATERS USED IN EXPERIMENTS

Water No.		Colour	Specific conductivity, μmho	pH	Alkalinity, as $CaCO_3$, p.p.m.	Total hardness, as $CaCO_3$, p.p.m.	Sulphate, p.p.m.	Chloride, p.p.m.
1	Nelspruit tap water	0 to 5	145	8·5	62	71	14	3
2	Boundary Creek	5 to 10	890	8·4	500	536	46	32
3	Buffelspruit	10 to 15	935	8·1	444	372	25	59
4	Nelsrivier	40	47	7·7	24	18	1	3
5	Borehole No. 1	5	66	7·3	30	20	1	4·5
6	Borehole No. 2	5	40	-	16	4·4	---	4·0

TABLE IV

DETERMINATION OF FLUORIDE IN VARIOUS WATERS

No.	Natural water	Optical density per 1 p.p.m. of fluoride	Optical density of sample	Fluoride found, p.p.m.	Fluoride added, p.p.m.	Optical density after adding fluoride	Fluoride found, p.p.m.	Actual fluoride, p.p.m.	Error, p.p.m. of fluoride
1	Compositions as in Table III	0·468	0·032	0·068	0·5	0·262	0·56	0·57	−0·01
2		0·413	0	0	0·5	0·206	0·50	0·50	0
3		0·392	0·077	0·20	0·5	0·254	0·65	0·70	−0·05
4		0·449	0·033	0·073	0·5	0·265	0·59	0·57	+0·02
5		0·442	0·078	0·18	0·5	0·292	0·66	0·68	−0·02
6		0·408	0·010	0·025	2·0	0·810	1·98	2·02	−0·04
3	containing 0·5 p.p.m. of aluminium	0·349	0·072	0·21	0·5	0·237	0·68	0·71	−0·03
5	containing 0·25 p.p.m. of aluminium	0·373	0·068	0·18	0·5	0·248	0·66	0·68	−0·02
6	containing 1·2 p.p.m. of iron*	0·398	0·012	0·030	2·0	0·810	2·04	2·03	+0·01
3	containing 1 p.p.m. of phosphate†	0·384	0·086	0·22	—	—	—	0·20	+0·02
3	containing 1 p.p.m. of phosphate† and 1 p.p.m. of fluorine	0·312	0·380	1·22	—	—	—	1·20	+0·02

* Added as $FeCl_3.6H_2O$.

† Phosphate was removed by precipitation with ferric chloride.

The optical density of the blank solution was, however, influenced by the presence of phosphate (see Table II). This influence was overcome by adding to the blank solution the same amount of phosphate as was present in the sample. If the amount of phosphate was not known, it was removed by neutralising 500 ml of the water with N hydrochloric acid or sodium hydroxide, with litmus paper as indicator. One millilitre of ferric chloride solution and then 2 ml of sodium acetate solution were added, and the solution was boiled and then filtered hot. When the filtrate was cool it was neutralised with N hydrochloric acid and made up to the original volume with distilled water.

ACCURACY—

The fluoride contents of 11 different waters were determined; the compositions of these waters are shown in Tables III and IV. A known amount of fluoride was added to each of the waters, and the total fluoride content was determined. The difference between the determined and calculated amounts showed that the error was not more than 0·05 p.p.m. of fluorine (see Table IV).

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The Absorptiometric Determination of Silicon in Water

Part I. Formation, Stability and Reduction of α- and β-Molybdosilicic Acids

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The effect of experimental conditions on the formation, stability (in the presence of reagents used for destroying molybdophosphoric acid) and reduction of α- and β-molybdosilicic acids has been determined. The effectiveness of several reagents for preventing interference from phosphate has also been investigated. Both α-molybdosilicic acid reduced by stannous tin and β-molybdosilicic acid reduced by 1-amino-2-naphthol-4-sulphonic acid, should be suitable for precise methods of determining "reactive" silicon in water.

At the steam pressures and temperatures used in modern power stations, silicic acid is appreciably soluble in steam. The concentration of silicon in boiler and make-up water is therefore controlled to ensure that the steam contains not more than about 0·02 p.p.m. of silica; above about 0·02 to 0·04 p.p.m. of silica undesirable deposits may be formed on turbine blades.

Methods are therefore needed for determining both the "reactive"* and total silicon concentrations in a range of aqueous samples from steam - water circuits in power stations. Thus the methods must be suitable for condensate, feed-water and steam (0·002 to 0·05 p.p.m. of silica), make-up water (0·005 to 0·2 p.p.m. of silica) and boiler water (0·2 to 75 p.p.m. of silica). Boiler waters often also contain added phosphate. The ratio of phosphate (p.p.m.) to silica (p.p.m.) is usually between 0 and 20, but occasionally ratios as high as 50 may occur.

The methods must also be precise, so that they can be used for determining small amounts of "non-reactive" silicon as the difference between the total and "reactive" silicon contents.

Absorptiometric methods in which the reduced α- and β-molybdosilicic acids are used appear to be the only sufficiently sensitive means of determining extremely small concentrations of silicic acid. They can also be used for determining total silicon provided all forms of silicon are converted to silicate or monomeric silicic acid in an earlier step.

This paper reports an investigation of the molybdosilicic acids to determine suitable conditions for each method; Parts II[2] and III[3] give details of the methods and the results obtained.

Two forms of molybdosilicic acid, α- and β-, are of analytical interest and were first distinguished by Strickland.[4] Each form can be reduced to several different blue compounds with various reducing agents, and Strickland[4] has determined the properties of several forms and the conditions for their formation. The relative amounts of α- and β-molybdosilicic acids produced and their stabilities appear to be affected by factors such as the concentrations of acids, molybdate, neutral salts and reagents such as oxalic or tartaric acid used for destroying molybdophosphoric acid. However, the literature does not give quantitative values for all these effects, and they have, therefore, been investigated.

EXPERIMENTAL

APPARATUS, REAGENTS AND TECHNIQUE—

All optical-density measurements were made in 4-cm cuvettes with a Hilger Uvispek spectrophotometer against distilled water in the reference cuvette. Measurements of pH were made with an E.I.L. type 23A pH meter, with glass and saturated-calomel electrodes.

A stock solution of sodium silicate was prepared by fusing 1·000 g of Specpure silicon dioxide (ignited at 1100° C) with 5 g of analytical-reagent grade sodium carbonate, dissolving the cooled melt in water, and diluting to 1 litre with distilled water. The silicon content was checked gravimetrically after two dehydrations with perchloric acid. Aliquots of this solution were diluted with distilled water to give solutions having the desired silicon concentrations. Distilled water from a Manesty still was used throughout; it contained 0·005 p.p.m. of silica or less and was stored in a polythene bottle. For factorial experiments, one large uniform batch of water was used in each experiment. The temperature of the laboratory varied between 20° and 25° C during this work.

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* "Reactive" silicon—mainly monomeric and dimeric silicic acid[1]—is defined in this paper as those forms of silicon that react with ammonium molybdate in 10 minutes to form molybdosilicic acid under the conditions of the method given in Part II of this series.

α-MOLYBDOSILICIC ACID

FORMATION—

Effect of pH—Strickland reported[4] maximum formation of α-molybdosilicic acid when silicic acid and molybdate were allowed to react at pH 3·7 to 4·0; formation was incomplete at higher pH values and β-molybdosilicic acid was formed at lower pH values. It was not clear from his paper whether the product in the pH range 3·7 to 4·0 would be entirely α-molybdosilicic acid at lower concentrations of silicon than he used. Preliminary work showed that this point could best be studied by reducing the product with stannous ions and measuring the optical densities of the solutions at the wavelengths of maximum absorption of the reduced α- and β-forms, *i.e.*, 742 and 810 mμ, respectively. The absorption spectra in Figs. 3 and 7 (see pp. 91 and 95) show that the optical-density ratio of the molybdosilicic acids (α to β) is 1·09 at 742 mμ and 0·414 at 810 mμ. Non-formation of either form would cause a decrease in optical density at both wavelengths.

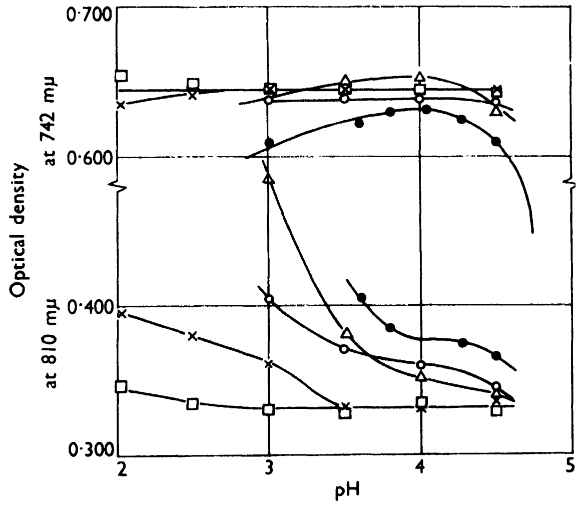

Fig. 1. Effect of pH on the formation of molybdosilicic acids: ●, 0·014 M MoO_4^{2-} (molybdate acidified before mixing with silicate); △, 0·028 M MoO_4^{2-} (molybdate acidified before mixing with silicate); ○, 0·014 M MoO_4^{2-} (silicate and molybdate mixed and then slowly acidified); X, 0·014 M MoO_4^{2-} (as for ○, but solution then heated in boiling water for 10 minutes); □, 0·014 M MoO_4^{2-} (as for ○, but solution then heated in boiling water for 20 minutes). The concentration of silicon was 0·5 p.p.m., as SiO_2; all concentrations were in the final solution

Fig. 1 shows the effect of various ways of combining solutions of ammonium molybdate, sodium silicate and hydrochloric acid. For these experiments, different amounts of ammonium molybdate in 50 ml of water were used; 5 ml of sodium silicate solution (10 p.p.m. of silica) were taken, and the solutions were acidified with dilute hydrochloric acid (1 + 200) to the desired pH. Ten minutes after the solutions had been mixed (except when they were heated), the acidity was increased by adding 15 ml of 17·5 N sulphuric acid, 2 ml of stannous oxalate reducing agent[5] were added, the volume was adjusted to 100 ml, and the optical densities were measured at 742 and 810 mμ.

The measurements at 810 mμ are most sensitive to changes in the ratio of α to β, and Fig. 1 shows that this ratio was markedly dependent on pH. However, the effect of pH was considerably decreased if solutions were heated before being reduced. Because of the pH-dependence of the unheated solutions, heating appeared to be a desirable step in an analytical method based on α-molybdosilicic acid, and the necessary heating period was accordingly determined.

Effect of period of heating—For these experiments, molybdosilicic acid was formed by adding 5 ml of silicate solution (10 p.p.m. of silica) to 5 ml of acidified 5 per cent. ammonium molybdate solution that had been diluted to 60 ml in a polythene bottle. The bottles were heated in a water bath for different periods and then cooled; 15 ml of 17·5 N sulphuric acid and, 1 minute later, 2 ml of 0·2 per cent. stannous reducing agent (as the chloride) were added. The solutions were then diluted to 100 ml, and the optical densities were measured at 742 and 810 mμ. The amount of acid added in the first stage was 10 ml of either 0·5 or 0·05 N sulphuric acid; the former probably corresponds to the worst possible conditions for producing α-molybdosilicic acid, as this acidity gives the maximum initial amount of the β-form (see section on β-molybdosilicic acid, p. 93). The latter acidity gives an initial pH of about 2·5, which is about the middle of the pH range for constant optical density when the solutions are heated (see Fig. 1).

Table I shows the period of heating after which constant optical densities were obtained at both wavelengths. It is considered probable that the constant optical densities correspond to the molybdosilicic acid being completely in the α-form.[4] For these tests, the silicon concentration used was near the upper concentration of interest; much larger concentrations may require longer heating periods.[6] Small variations in the concentrations of ammonium molybdate are not expected to have any large effect on the rate of conversion of the β- to the α-form.[6] In all further work in which solutions of pure α-molybdosilicic acid were required (except when otherwise stated), they were prepared as described above, 10 ml of 0·05 N sulphuric acid being used, and heating for 60 minutes in boiling water.

TABLE I

HEATING PERIOD REQUIRED TO GIVE 100 per cent. α-MOLYBDOSILICIC ACID

Final concentrations of silicate and ammonium molybdate were 0·5 p.p.m. of silica and 0·014 M MoO_4^{2-}, respectively

Sulphuric acid added initially,		Period of heating required to give constant optical densities at water bath temperatures of—	
ml	N	80° C, minutes	100° C, minutes
10	0·5	60	30
10	0·05	15	Not determined

Stability and absorption curve—The reported stability of α-molybdosilicic acid[4,6] was confirmed; the optical density at 430 mμ of unreduced solutions, prepared as described above, was constant to within ±2 per cent. for at least 72 hours. The absorption curve, between 370 and 450 mμ, was similar to those reported by other workers.[4,6]

EFFECT OF TARTARIC, OXALIC OR HYDROCHLORIC ACID—

For these tests, different amounts of the acids were added to identical solutions of α-molybdosilicic acid, prepared by slow acidification of a solution containing silicate and ammonium molybdate (see Fig. 1), and the optical densities of the solutions were measured at 430 mμ over a period of 60 minutes. The rates of decrease in optical density during the first 15 minutes were approximately constant and are shown in Fig. 2. Because of the method of preparation used, the α-molybdosilicic acid probably contains about 10 per cent. of the β-form, and the curves for the α-form will be somewhat high.

REDUCTION—

By stannous tin—Milton showed[7] that stannous chloride could be successfully used for reducing α-molybdosilicic acid in 2 N sulphuric acid. Our experiments showed that reducing α-molybdosilicic acid with stannous tin in the presence of 2·5 N hydrochloric acid gave an extremely unstable product. The optical density varied from experiment to experiment and decreased rapidly when the solution was allowed to stand. By carrying out the reduction in 2·5 N sulphuric acid, with only 0·0033 N chloride present, an intense blue-green colour, stable for an hour, could be produced. The reducing agent was prepared by dissolving 0·1 g of tin foil in 1·5 ml of concentrated hydrochloric acid and diluting to 50 ml with water.

The absorption curve of this stable reduced product is shown in Fig. 3. The spectrum below 550 mμ was not reproducible and changed with time and the concentration of reducing agent. This part of the spectrum is probably caused by reduction of the excess of molybdate, as a similar curve was also obtained with reagent blank solutions. The absorption spectrum

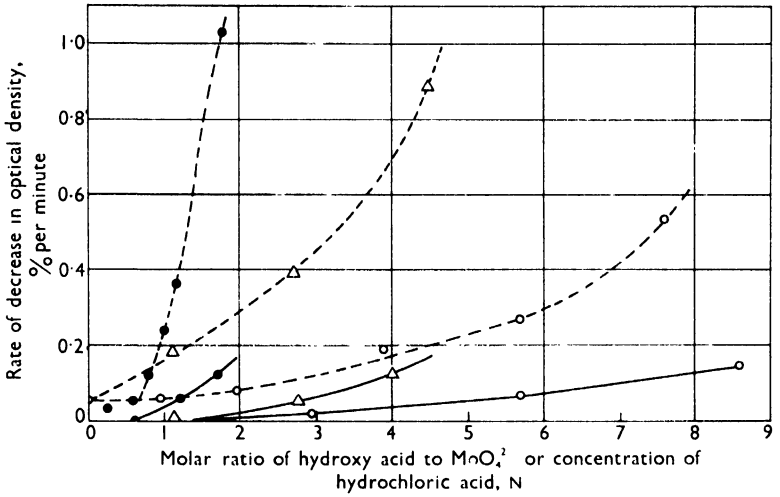

Fig. 2. Destruction of α- (full lines) and β- (broken lines) molybdosilicic acids by acids: ●, oxalic acid; △, hydrochloric acid; ○, tartaric acid. The concentrations of silicon and molybdate in the final solution were 5 p.p.m., as SiO_2, and 0·014 M MoO_4^{2-}, respectively

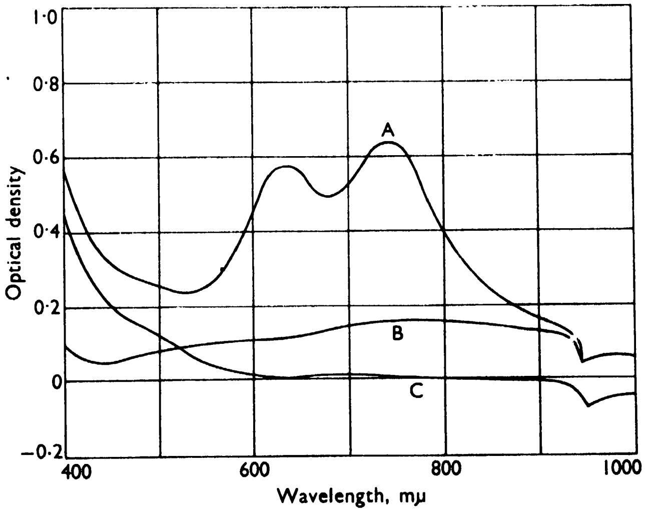

Fig. 3. Absorption spectra of α-molybdosilicic acid reduced by: curve A, stannous tin in 2·5 N sulphuric acid; curve B, 1-amino-2-naphthol-4-sulphonic acid; curve C, reagents only, reduced by stannous tin in 2·5 N sulphuric acid. The concentrations of silicon and molybdate in the final solution were 0·5 p.p.m., as SiO_2, and 0·014 M MoO_4^{2-}, respectively

is similar to that obtained by Strickland[4] for the +4e reduced compound, except for the peak at 635 mμ, which shows on Strickland's curve as a flat portion at 650 mμ. Strickland's curve was measured in the absence of excess of molybdate, and the α-molybdosilicic acid was prepared in a different manner from that used in this work. Fig. 3 shows that, for analytical use, the most suitable wavelengths for optical-density measurements are 742 mμ and 635 mμ. The former is preferred because, although the peak heights are much the same, the optical density of the blank solution is less at this wavelength.

The effects of light and the concentrations of stannous tin and chloride on the stability of the reduced α-molybdosilicic acid are shown in Figs. 4 and 5; the coefficient of variation of the optical densities was about ±0·1 per cent. The figures show, therefore, that the final concentration of stannous tin should be 0·34 × 10^{-3} M and that of the chloride less than 0·008 M, if the optical densities of the reduced solutions are to be constant for one hour. Further, the solutions should be kept out of direct sunlight.

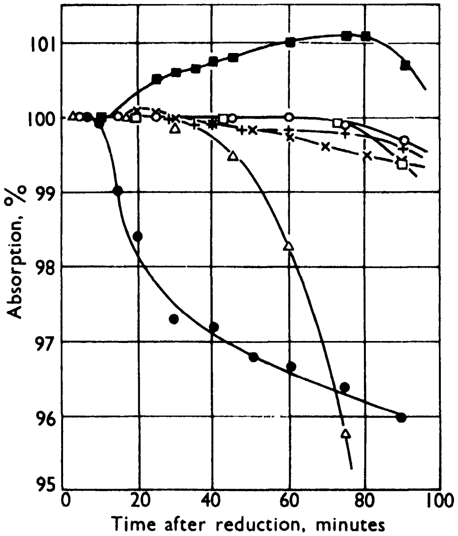

Fig. 4. Effect of stannous tin and chloride on the stability of reduced α-molybdosilicic acid: ●, 0·085 × 10^{-3} M Sn^{2+}, 0·0033 M Cl^-; X, 0·17 × 10^{-3} M Sn^{2+}, 0·0033 M Cl^-; +, 0·25 × 10^{-3} M Sn^{2+}, 0·0033 M Cl^-; ○, 0·34 × 10^{-3} M Sn^{2+}, 0·0033 M Cl^-; ■, 0·68 × 10^{-3} M Sn^{2+}, 0·0033 M Cl^-; □, 0·34 × 10^{-3} M Sn^{2+}, 0·0083 M Cl^-; △, 0·34 × 10^{-3} M Sn^{2+}, 0·028 M Cl^-. All concentrations were in the final solution

In the experiments described above, reduction took place in 2·5 N acid. This involved adding a large volume of acid, which raised the temperature of the test solution. Some tests were therefore made to determine whether or not tartaric acid could be used in place of sulphuric acid. When α-molybdosilicic acid was reduced with stannous tin in the presence of 0·053 M tartaric acid, a blue colour resulted that remained stable for 20 minutes and then slowly faded. The absorption spectrum initially resembled that of Strickland's +5e compound,[4] but after 90 minutes it had changed to that of the +4e compound. Thereafter, the spectrum remained in this form as the optical density of the solution continued decreasing. Strickland showed that strongly acidified molybdate instantly oxidised the +5e compound to the +4e form; the +5e form is, therefore, not produced in the presence of excess of acidified molybdate. However, the experiment just described shows that, in the presence of tartaric acid, which complexes the molybdate ion, a considerable amount of +5e form is produced. Thus, the reduction of α-molybdosilicic acid by stannous tin in the presence of tartaric acid is unsuitable for the basis of an analytical method.

By 1-amino-2-naphthol-4-sulphonic acid—Preliminary experiments showed that the optimum concentration of 1-amino-2-naphthol-4-sulphonic acid for reducing α-molybdosilicic acid in the presence of 2·5 N sulphuric acid was about 0·004 per cent. The absorption spectrum of this reduced product in the presence of 2·5 N sulphuric acid is shown in Fig. 3. The curve in the presence of 0·053 M tartaric acid had the same shape, but the optical density was about 0·004 to 0·008 lower at most wavelengths. Because of the low absorption, compared with that given by stannous tin, no further work was done with this reducing agent.

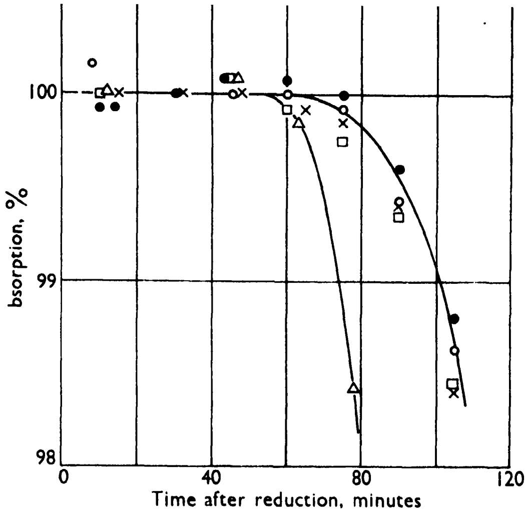

Fig. 5. Effect of light on the stability of reduced α-molybdosilicic acid: ○, liquid kept in flask in the dark; □, liquid kept in flask in a light room out of sunlight; △, liquid kept in flask in sunlight; ●, liquid kept in a cell in the dark; X, liquid kept in a cell in the light path of a spectrophotometer

β-MOLYBDOSILICIC ACID

FORMATION—

Factors affecting the formation of β-molybdosilicic acid—Strickland showed[4] that the ratio of H+ to MoO_4^{2-} is the most important factor governing formation of β-molybdosilicic acid; the optimum ratio lies between 3 and 5. He did not show that his product consisted entirely of the β-form, and possibly there is always some α-molybdosilicic acid present. If the β-form is to be used in an analytical method, it is desirable that the amount produced (from a given concentration of silicic acid) should not depend critically on experimental conditions. Reducing molybdosilicic acid with 1-amino-2-naphthol-4-sulphonic acid gives a sensitive indication of the relative amounts of α- and β-forms present in a solution; at 810 mμ, the absorption ratio, α to β, is 0·2 (see Figs. 3 and 7).

The effects produced by changes in certain experimental factors were therefore tested. For all these experiments, the H+ to MoO_4^{2-} ratio was 4·5 and reduction was carried out with 1-amino-2-naphthol-4-sulphonic acid. A ratio of 4 was not used, because an acidified molybdate reagent with such an acidity often develops a blue colour on standing. Although this colour is destroyed by the tartaric acid usually added, it may be avoided by increasing the acidity to give a H+ to MoO_4^{2-} ratio of 4·5. In the standard procedure (see Table II) 2·5 ml of acidified ammonium molybdate solution (8·9 per cent. w/v) were added to 50 ml of water containing 5 ml of silicate solution containing 15 p.p.m. of silica. After 10 minutes, sufficient tartaric acid solution (28 per cent. w/v) was added to make the molar ratio, tartaric acid to MoO_4^{2-}, 3·6, and 5 minutes later 2 ml of 0·2 per cent. 1-amino-2-naphthol-4-sulphonic acid solution (containing 2·4 per cent. of hydrated sodium sulphite and 14 per cent. of anhydrous potassium metabisulphite) were added. The solution was diluted to 100 ml with water, and the optical density was measured at 810 mμ. The results of duplicate measurements (corrected for reagent blank values) are shown in Table II, which indicates that any effects produced by the factors tested were small.

TABLE II

FORMATION OF β-MOLYBDOSILICIC ACID UNDER VARIOUS CONDITIONS

All concentrations are in the final solution

Level of the variable factor		Optical density compared with that for the standard conditions, per cent.
Standard conditions*		100·0 (taken as standard)
0·05 M MoO_4^{2-}		102·7, 102·0 (mean 102·4)
0·05 M MoO_4^{2} and 50 p.p.m. of PO_4^{3-}		99·3, 99·5 (mean 99·4)
0·0125 M MoO_4^{2-} and 50 p.p.m. of PO_4^{3-}		98·6, 98·4 (mean 98·5)
Initial volume of sodium silicate solution, ml	7·5	98·7, 98·3 (mean 98·5)
	10	99·0, 99·2 (mean 99·1)
	30	100·9, 100·2 (mean 100·6)
	65	99·6, 99·8 (mean 99·7)
	85	99·4, 99·5 (mean 99·4)
Temperature during formation of β-molybdosilicic acid	15° C	99·9, 100·2 (mean 100·0)
	30° C	99·1 (mean 99·1)
Reversed procedure, *i.e.*, 5 ml of sodium silicate added to 2·5 ml of ammonium molybdate reagent, diluted to 50 ml with water		99·3, 99·2 (mean 99·2)

* Standard conditions: 0·75 p.p.m. of silica; 0·0125 M MoO_4^{2} ; 0 p.p.m. of phosphate; initial volume 50 ml; temperature 21° C; acidified ammonium molybdate solution added to the sodium silicate solution.

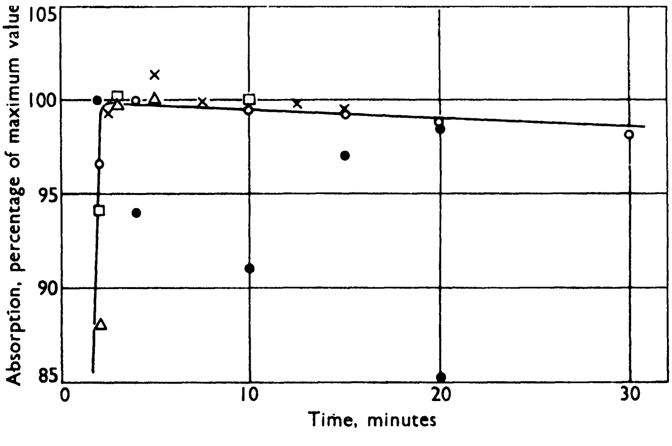

Fig. 6. Change in absorption of final solution with time allowed for formation of β-molybdosilicic acid: X, 0·05 M MoO_4^{2-}, 0·5 p.p.m. of SiO_2 and 25 p.p.m. of PO_4^{3-}; ●, 0·0125 M MoO_4^{2} and 0·01 p.p.m. of SiO_2; ○, 0·0125 M MoO_4^{2-} and 0·2 p.p.m. of SiO_2; □, 0·0125 M MoO_4^{2-} and 0·44 p.p.m. of SiO_2; △, 0·0125 M MoO_4^{2-}, 0·44 p.p.m. of SiO_2 and 44 p.p.m. of PO_4^{3-}. All concentrations were in the final solution

Period required for formation of β-molybdosilicic acid—The length of time required to allow complete formation of β-molybdosilicic acid was determined by varying the period between adding the molybdate and tartaric acid reagents; all other conditions were similar to those for the standard procedure described above, except that the volume of samples *plus* acidified molybdate reagent had different values, between 75 and 102·5 ml, in the various experiments. The experiments were made at different silicon concentrations and, in order to show all the results on one curve, they are given in Fig. 6 as percentages of the maximum optical density for each set of conditions. The figure shows that the optical density had always reached a maximum value in 3 minutes.

The figure also confirms the reported[4] decrease in absorption caused by conversion of β-molybdosilicic acid to the α-form. Salts have been reported[4] to accelerate the conversion, and this effect was investigated by measuring the optical densities of unreduced β-molybdosilicic acid over a period of time; the concentration of ammonium molybdate was 0·028 M MoO_4^{2-} in the final solution for these tests. At a silicon concentration of 5 p.p.m., as SiO_2, the rate of decrease in optical density (at 430 mμ) was 0·05 per cent. per minute and 0·06 per cent. per minute in the presence of 0·34 M (20,000 p.p.m.) sodium chloride.

Absorption curve—The absorption curve between 370 and 500 mμ was similar to those given in the literature[4] for β-molybdosilicic acid.

EFFECT OF TARTARIC, OXALIC OR HYDROCHLORIC ACID—

Fig. 2 shows these effects, which were determined in a similar manner to those for α-molybdosilicic acid. No correction has been made for the change of β-molybdosilicic acid to the α-form.

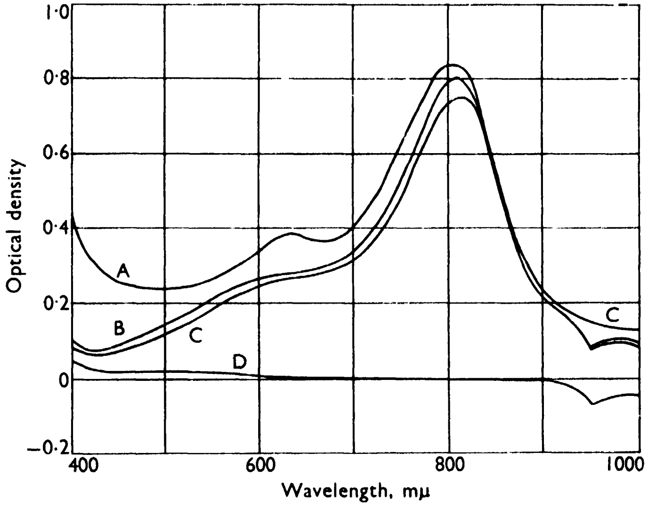

Fig. 7. Absorption spectra of β-molybdosilicic acid reduced by: curve A, stannous tin in 2·5 N sulphuric acid; curve B, 1-amino-2-naphthol-4-sulphonic acid in 2·5 N sulphuric acid; curve C, 1-amino-2-naphthol-4-sulphonic acid in 0·053 M tartaric acid; curve D, reagents only, reduced by 1-amino-2-naphthol-4-sulphonic acid in 0·053 M tartaric acid. The concentrations of silicon and molybdate in the final solution were 0·483 p.p.m., as SiO_2, and 0·014 M MoO_4^{2-}, respectively

REDUCTION—

By 1-amino-2-naphthol-4-sulphonic acid—Fig. 7 shows the absorption curves of β-molybdosilicic acid reduced by 1-amino-2-naphthol-4-sulphonic acid under different conditions. The shapes of the curves are similar to those given in the literature for analogous solutions. The figure shows that the absorption in 2·5 N sulphuric acid is greater than that in tartaric acid. This increased absorption was found whether sulphuric acid was added before or after reducing the molybdosilicic acid and is, therefore, probably caused by increased absorption of light by β-molybdosilicic acid in solutions of high acidity. Reduced and unreduced α-molybdosilicic acid gave a similar effect.

The optical density at 810 mμ was constant within ±0·14 per cent. over a period between 15 minutes and 24 hours after reduction, when the final concentration of 1-amino-2-naphthol-4-sulphonic acid was 0·17 or 0·34 × 10^{-3} M (0·004 or 0·008 per cent.); at lower concentrations than about 0·04 × 10^{-3} M the optical density increased slowly over at least an hour and, at higher concentrations than 0·34 × 10^{-3} M, crystals of 1-amino-2-naphthol-4-sulphonic acid formed in the solution. Between 0·34 and 0·042 × 10^{-3} M, the maximum optical density increased steadily with decrease in concentration; it was 0·8 to 2·4 per cent. more at 0·042 than at 0·34 × 10^{-3} M, and the results were unaffected by 25 p.p.m. of phosphate.

By stannous tin—Fig. 7 shows the absorption spectrum of β-molybdosilicic acid reduced by stannous tin in 2·5 N sulphuric acid. The small peak at 635 mμ was not found in 2·5 N hydrochloric acid, nor was it shown on Strickland's curve,[4] which was measured in 1 N hydrochloric acid. Fig. 7 also shows that the absorption was greater than that obtained when 1-amino-2-naphthol-4-sulphonic acid was used; the difference is not large, but the curves have a slightly different shape. Two possible explanations could account for this difference. Either different reduced states of β-molybdosilicic acid are produced by stannous tin and 1-amino-2-naphthol-4-sulphonic acid or variable amounts (about 20 per cent.) of α-molybdosilicic acid are present in the supposedly pure β-form. There is evidence for both possibilities, and it should be possible to decide between them by accurately determining the number of electrons, per molecule of molybdosilicic acid, involved in the reduction by the two reagents. This has not been done in the work described here.

In the presence of 0·004 per cent. of stannous tin (the optimum concentration for stability of the reduced β-molybdosilicic acid) and 2·5 N sulphuric acid, the optical density of the final solution decreased by about 0·4 per cent. in the first hour; the decrease was somewhat erratic from experiment to experiment.

DESTRUCTION OF MOLYBDOPHOSPHORIC ACID

The previous sections have shown that α-molybdosilicic acid is best reduced by 0·004 per cent. stannous tin in the presence of 2·5 N sulphuric acid and β-molybdosilicic acid by 0·004 per cent. 1-amino-2-naphthol-4-sulphuric acid in the presence of tartaric or oxalic acids. The effect of these two sets of conditions on the stability of molybdophosphoric acid was therefore determined.

UNDER CONDITIONS FOR PRODUCING α-MOLYBDOSILICIC ACID—

The standard procedure was as described below. An 8-ounce polythene bottle (containing 50 ml of water, 5 ml of 5 per cent. ammonium molybdate solution, 10 ml of 0·05 N hydrochloric acid and the desired amount of phosphate) was placed in boiling water for one hour. The solution was set aside to cool, and then 15 ml of 17·5 N sulphuric acid and, 1 minute later, 2 ml of 0·2 per cent. stannous (as chloride) solution were added. The solution was then cooled to 22° C, diluted to 100 ml, and its optical density measured at 742 mμ within one hour. Phosphate was added as ammonium phosphate, prepared by passing 1 N ammonium hydroxide solution through a column of Amberlite IR45 anion-exchange resin in the phosphate form. When this solution was diluted to give a phosphate concentration of 25 p.p.m., the silicon content of the diluted solution was less than 0·001 p.p.m., as SiO_2 (as determined by a β-molybdosilicic acid method).

In the presence of 25 p.p.m. of phosphate a variable optical density was obtained, equivalent to 0·02 to 0·08 p.p.m. of silica, in the presence or absence of silicon; smaller concentrations of phosphate had a larger effect per p.p.m. of phosphate. The effect was not reduced by increasing the period of the high-acidity stage, but, when the solutions were not heated, the effect was equivalent to only 0·005 p.p.m. of silica. These results, therefore, show that the substance causing the effect was formed mainly during the heating stage and was not easily destroyed by 2·5 N sulphuric acid. The substance is probably a fine precipitate of molybdophosphoric acid (see next section), and the method is therefore not particularly suitable for samples containing much phosphate. For the maximum effect found, 1 p.p.m. of phosphate was equivalent to 0·008 p.p.m. of silica.

UNDER CONDITIONS FOR PRODUCING β-MOLYBDOSILICIC ACID—

To determine the effect of the concentration of tartaric or oxalic acids, the experiment described below was carried out. To 85 ml of water containing 118 p.p.m. of phosphate (as Na_2HPO_4) were added 4 ml of acidified molybdate solution (ratio H^+ to MoO_4^{2-} = 4·5). Ten minutes later, 4 ml of either tartaric acid or oxalic acid solution were added, and, after a further 5 minutes, 4 ml of 0·094 per cent. 1-amino-2-naphthol-4-sulphonic acid solution were added, and the optical density was then measured at 810 mμ. The results (corrected for reagent blank values) are shown in Fig. 8 and were used to choose a suitable value for the molar concentration ratio, hydroxy acid to MoO_4^{2-}, to be used in the next experiment.

To determine the rate of destruction of molybdophosphoric acid by these two hydroxy acids, some tests were carried out exactly as described above, except that the time between adding the hydroxy acid and the 1-amino-2-naphthol-4-sulphonic acid was varied. The results (corrected for reagent blank values) are shown in Fig. 9. The figure shows that, when a suitable amount of hydroxy acid was used, most of the molybdophosphoric acid was destroyed within 4 minutes, although there was a small residual absorption.

The residual optical density is apparently caused by a fine yellow precipitate (probably molybdophosphoric acid), which, under some conditions, is heavy and may cause serious interference. Important factors governing its formation (at a H^+ to MoO_4^{2-} ratio of 4 to 4·6)

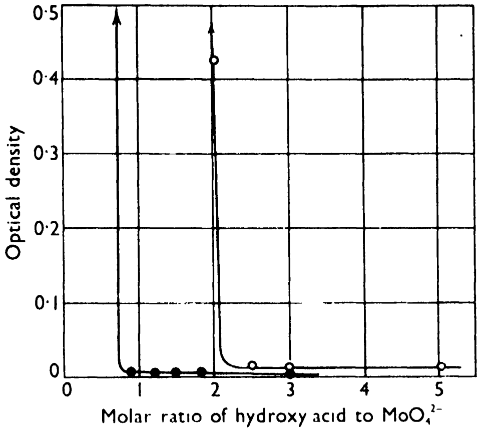

Fig. 8. Effect of acids on molybdophosphoric acid: ○, tartaric acid; ●, oxalic acid. The concentrations of molybdate and phosphate in the final solution were 0·0125 M MoO_4^{2-} and 103 p.p.m. of PO_4^{3-}, respectively. The interval between adding hydroxy acid and reducing agent was 5 minutes

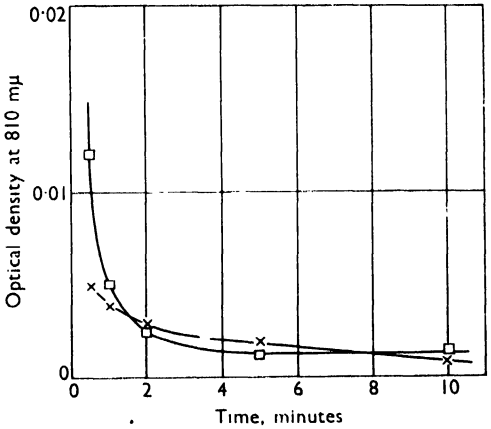

Fig. 9. Variation in residual absorption with time allowed for destruction of molybdophosphoric acid by hydroxy acids: □, tartaric acid, 103 p.p.m. of PO_4^{3-} and 0·0125 M MoO_4^{2-}; X, oxalic acid, 103 p.p.m. of PO_4^{3-} and 0·0125 M MoO_4^{2-}. The ratio of tartaric acid to MoO_4^{2-} was 3·5 and of oxalic acid to MoO_4^{2-} was 2·0

are the concentrations of phosphate and molybdate and the history of the flasks in which the reaction is carried out ("seeding" effect); other factors have not been investigated. "Seeding" effects were detected because successive determinations in the same flask showed increasing interference from phosphate; the effect could be overcome by washing the flasks with 2 N ammonium hydroxide solution.

At a molybdate concentration in the final solution of 0·05 M, no seeding effect was detected in the presence of 10 p.p.m. of phosphate (as Na_2HPO_4) in the final solution after seven determinations in each of two flasks (interference effect, 1 p.p.m. of phosphate ≡ 0·0002 to 0·0019 p.p.m. of silica at 810 mμ). With 50 p.p.m. of phosphate, seeding occurred after one determination (initial effect, 1 p.p.m. of phosphate ≡ 0·0008 p.p.m. of silica) and with 100 p.p.m. of phosphate a heavy precipitate formed during the first determination in a flask. When the concentration of molybdate in the final solution was 0·0125 M, 50 p.p.m. of phosphate did not cause a seeding effect after 5 determinations (interference effect, 1 p.p.m. of phosphate ≡ 0·0000 to 0·0002 p.p.m. of silica at 810 mμ) and 100 p.p.m. of phosphate did not cause a precipitate. At 670 mμ the interference effects were 2·5 times greater than those given above. It appears, therefore, that for samples containing much phosphate, it is preferable to use 0·0125 M MoO_4^{2-} in the final solution rather than 0·05 M.

PREVENTION OF THE REDUCTION OF MOLYBDATE

When the acidity of ammonium molybdate solutions is increased to 2·5 N, the molybdate apparently changes to a form that is not reduced by stannous tin to a blue compound; this change might be expected to require a finite time. The effect, on the reagent blank value, of the time between adding acid and reducing agent was therefore determined. Experimental conditions were the same as the standard conditions given in a previous section for α-molybdosilicic acid. When the initial volumes of solution were 30, 50 and 60 ml, no significant effect, compared with the standard deviation for such determinations (0·0007 optical-density units), was found for times between 15 and 60 seconds. The mean optical density of the reagent blank solutions was 0·003.

EFFECT OF TEMPERATURE ON THE ABSORPTION OF LIGHT BY THE REDUCED MOLYBDOSILICIC ACIDS

The magnitude of this effect was estimated by placing warm solutions in the spectrophotometer, measuring first the temperature with a mercury thermometer and then the optical density. This was repeated as the solution cooled.

The optical densities of solutions of α-molybdosilicic acid reduced by stannous tin increased by about 0·15 per cent. per °C decrease in temperature. The corresponding figure for β-molybdosilicic acid reduced by 1-amino-2-naphthol-4-sulphonic acid was about 0·25 per cent. per °C.

DISCUSSION OF THE WORK

The lowest concentration of silicon of interest in samples was about 0·002 p.p.m., as silica. The limit of detection of a method will be determined by the reproducibility of the reagent blank value, and, if the spectrophotometric sensitivity of the absorbing substance is low, this limit may, in practice, be determined by the reproducibility of the spectrophotometer. As the unreduced molybdosilicic acids have much lower spectrophotometric sensitivities[4] than the reduced forms, except in the ultraviolet part of the spectrum in which the reagent blank solutions also absorb strongly, only the use of the reduced acids was further considered.

The work described above shows that a method based on reduced α-molybdosilicic acid may be expected to give precise results, because: (1) the acid can easily be produced in constant amount, (2) it is little affected by the high acidity at which reduction is carried out, (3) the reduced product is stable for at least an hour and (4) there appear to be no particularly critical factors to be controlled. An advantage of such a method is the reported insensitivity to high concentrations of salts. Its chief disadvantages are: (*a*) the heating operation, which adds to the necessary time and number of manipulations, (*b*) measurements must be completed within an hour after adding the reducing agent and (*c*) the sensitivity to phosphate and chloride interference.

Those factors that might make reduced β-molybdosilicic acid unsuitable for use in a precise method have been shown to be less important than expected from the literature. Thus—in the absence of large concentrations of salts at least—β-molybdosilicic acid can easily be prepared in constant amount, and its conversion to the α-form seems insufficiently fast to be a source of large errors. Care is needed in choosing a reagent to destroy molybdophosphoric acid. When sufficient oxalic or tartaric acid is used, the rate of destruction of molybdophosphoric acid and the residual absorption caused by phosphate were similar for both reagents. However, the rate of destruction of molybdosilicic acid is much faster with oxalic than with tartaric acid, and the latter is therefore preferred. As a reducing agent, 1-amino-2-naphthol-4-sulphonic acid is considered superior to stannous tin, because of the greater stability of the reduced product; the slightly greater sensitivity given by stannous tin seems insufficient to warrant its use. When suitable conditions are used, a method based on reduced β-molybdosilicic acid should give reproducible results, can be made insensitive to phosphate interference and does not require many operations. The major disadvantage is the reported[4] interference of large amounts of neutral salts.

The spectrophotometric sensitivities[8] of the reduced α- and β-molybdosilicic acids are 0·0030 μg of silica per sq. cm at 742 mμ and 0·0026 μg of silica per sq. cm at 810 mμ, respectively. When a spectrophotometer is not available and a filter giving a peak at about 670 mμ (*e.g.*, Ilford No. 608) is used, the sensitivities change to 0·0039 μg of silica per sq. cm and 0·0069 μg of silica per sq. cm for the α- and β-forms, respectively.

CONCLUSIONS

Both reduced α- and β-molybdosilicic acids should be suitable as the bases of precise methods for determining "reactive" silicon in water. Many of the potential sources of error have been evaluated and can be minimised by using appropriate conditions as indicated in this paper. Such methods should be further tested to determine the attainable precision, sensitivity to variations in conditions and liability to interference. These further tests for two methods involving the use of the α-[3] and β-forms[2] will be described in later papers of this series.

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The Absorptiometric Determination of Silicon in Water

Part II. Method for Determining "Reactive" Silicon in Power-station Waters

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A method, together with a modification for obtaining high sensitivity, is described for determining the "reactive" silicon content of water; it is based on the absorptiometric measurement of solutions of reduced β-molybdosilicic acid. The within-batch coefficient of variation of the optical-density difference, optical density of sample less optical density of reagent blank solution, varied from 7 per cent. to 0·2 per cent. for concentrations of 0·01 and 0·5 p.p.m. of silica, respectively. The limit of detection was about 0·001 p.p.m. of silica. The effects caused by several other substances have been determined. The analysis time is about 1½ hours for a batch of 10 samples in duplicate.

A METHOD was required for determining "reactive"* silicon in water in the range 0·002 to 75 p.p.m. of silica. The types of samples to be analysed were (*a*) de-ionised water, feed water, make-up water and steam, which usually contain less than 1 p.p.m. of silica, and (*b*) boiler water, containing phosphate, alkali and between 0·2 and 75 p.p.m. of silica.

From the work described in Part I of this series[1] it appeared that a suitable method could be based on the absorptiometric measurement of β-molybdosilicic acid, after reduction by 1-amino-2-naphthol-4-sulphonic acid. Desirable experimental conditions were therefore chosen, and this paper describes the procedure together with the tests performed to confirm its suitability for use with de-ionised water, feed-water, make-up water and steam. It is likely that the method will also be suitable for boiler-water, but it has not yet been tested sufficiently for a definite recommendation to be made; it is hoped that this part of the work will be reported later.

METHOD

REAGENTS—

All reagents should be of analytical grade unless otherwise stated. Silicon in the tartaric acid solution and in reagents added after it has no affect on the final absorption. Other reagents, including water, should be stored in polythene vessels.

Water—Water used for preparing reagents and for diluting samples should preferably contain less than about 0·005 p.p.m. of silica, as determined by the high-sensitivity modification of the method; if the silicon concentration is higher, reagent blank values for the high-sensitivity modification may sometimes cause appreciable bias because of silicon in the 5 ml of water used in this blank solution. Distilled water from an all-metal still, or water that has been passed through a mixed-bed de-ionisation unit, was found to be suitable.

Acidified molybdate solution—Dissolve 89 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in about 800 ml of water at room temperature. Dilute 62 ml of 98 per cent. sulphuric acid to about 100 ml by adding it cautiously to water, with stirring, and allow to cool. Add the acid to the molybdate solution, and dilute to 1 litre. The reagent may be kept for several months. Any slight blue colour developing in this reagent may be ignored unless the reagent blank values become unduly large.

Tartaric acid solution, 28 per cent. w/v—The reagent may be kept for at least 3 months.

Reducing agent solution—Dissolve 2·4 g of sodium sulphite, $Na_2SO_3.7H_2O$, and 0·2 g of 1-amino-2-naphthol-4-sulphonic acid (purest grade available) in about 70 ml of water. Add 14 g of potassium metabisulphite, shake well until dissolved, and dilute to 100 ml. This reagent should be freshly prepared each week.

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* "Reactive" silicon is defined in this paper as those forms of silicon—mainly monomeric and dimeric silicic acid—that react with ammonium molybdate in 10 minutes under the conditions of the method given in this paper.

Standard solutions of silica—Fuse 1·000 g of pure dry silica with 5 g of anhydrous sodium carbonate in a platinum crucible at red heat. When cool, dissolve in water, and dilute to exactly 1 litre. This solution contains 1000 p.p.m. of silica. Prepare by dilution a solution containing 10 p.p.m. of silica. The solutions containing 1000 p.p.m. were stable, within ±0·5 per cent., for at least 2 years in polythene bottles, and the solutions containing 10 p.p.m. for at least 3 months.

The most suitable silica for this purpose is probably transparent Spectrosil rod (Thermal Syndicate Ltd.), which has metallic impurities of less than 1 p.p.m. and is not appreciably hygroscopic.

PROCEDURE—

By pipette, place in a 100-ml calibrated flask sufficient sample to give a suitable optical density, and dilute to 80 ± 5 ml with water; in a similar flask for the reagent blank solution place the same amount of water as was used for diluting the sample.

Add 2·5 ml of acidified molybdate solution to the contents of both flasks, and mix immediately. Ten minutes ±3 minutes later add 2·5 ml of tartaric acid solution, and mix immediately. Five minutes ±1 minute after the tartaric acid has been added, add 2·0 ml of reducing agent solution, mix, and dilute to exactly 100 ml. Between 15 minutes and 24 hours later (1 hour if the samples contain phosphate), measure the optical densities in a suitable cuvette (preferably 4-cm) against distilled water. Subtract the optical density of the reagent blank solution from that of the sample, and read off the concentration of silicon from the calibration curve.

The optical density is best measured at 810 mμ (the peak is sharp and should be checked on each instrument); with 4-cm cuvettes, 0·67 p.p.m. of silica in the final solution has an optical density of about 1·0. The optical density can also be measured at about 670 mμ (*e.g.*, with Ilford No. 608 filters), but the reading is only 40 per cent. of that at 810 mμ; 10-cm cuvettes can then be used to increase the sensitivity.

Preparation of calibration curve—Treat as samples suitable aliquots of standard silicon solution containing 10 p.p.m. of silica; the blank solution should contain 80 ml of water from the same batch as was used for preparing and diluting the standard silicon solutions. From the results obtained prepare a calibration graph.

Compensation for turbidity and/or colour in a sample—A blank solution often suitable for compensating for the turbidity and/or colour of samples can be prepared by adding to a normally prepared blank solution, after the tartaric acid has been added, the same volume of sample that was used in the determinations. A check should then also be made on whether the turbidity and/or colour is differently affected by the sample and blank procedures.

MODIFICATION FOR HIGHEST SENSITIVITY—

By pipette, place a 100-ml sample in a 4-ounce polythene bottle; place 5 ml of water in a similar bottle for the reagent blank solution. With the same timing as in the basic procedure, add the same volumes of reagents to the contents of the bottles, except that, after adding tartaric acid to the reagent blank solution, add 95 ml of water to this bottle. Do not dilute the solutions any further before measuring their optical densities, preferably at 810 mμ in 10-cm cuvettes, against distilled water. Subtract the optical density of the reagent blank solution from that of the sample, and read off the concentration of silicon from a calibration curve. This is prepared by treating 100-ml aliquots of suitable silicon solutions exactly as for samples. For this curve, the blank determination should be made in exactly the same way as for samples, *i.e.*, 100 ml of water (from the same batch as used for preparing and diluting the silicon solutions) should be taken initially.

SOURCES OF ERROR—

Reagent blank solutions prepared in borosilicate flasks contained only 0·0005 to 0·0015 p.p.m. more of silica than did those prepared in polythene bottles, provided (1) flasks were reserved for these analyses, (2) old solutions were left in them until just before the next determination and (3) they were rinsed shortly before use. However, two flasks gave systematically high blank values, and flasks should therefore be checked before first being used for analyses. Just before use, pipettes should be rinsed with the solution to be dispensed.

If techniques are chosen so that the maximum error involved in each of (*i*) weighing chemicals, (*ii*) adjusting reagent solutions to volume and (*iii*) dispensing the required volume of reagent solution, is less than ±5 per cent., errors from these sources should be extremely small (<1 per cent.).

RESULTS

VARIATION IN CONCENTRATION OF REAGENTS—

The effect of relatively small variations in the concentrations of ammonium molybdate, sulphuric acid and tartaric acid was determined in a factorial experiment. Three concentration levels were used for each reagent: standard, 20 per cent. less than standard and 20 per cent. more than standard. Other conditions were as described in the procedure for the high-sensitivity modification. Single determinations were carried out for each treatment, and the design and analysis of the experiment were the same as those described by Davies.[2] The experiment was carried out at a silicon concentration of 0·01 p.p.m., as silica, in the sample and repeated at a concentration of 0·2 p.p.m., as silica.

Analysis of the results led to the conclusions: (1) that at 0·01 p.p.m. of silica, variation in the reagents had no significant effect, compared with a standard deviation for random errors of ±0·00075 p.p.m. of silica; (2) that at 0·2 p.p.m. of silica, variation in the concentration of sulphuric acid had no significant effect, but a ±20 per cent. change in the concentration of either ammonium molybdate or tartaric acid gave a ±0·6 per cent. change in optical density; the maximum error from this source was, therefore, ±1·2 per cent.

The effect of a ±20 per cent. variation in the concentration of the reducing agent was known, from the work reported in Part I of this series, to be no more than ±0·2 per cent.

LINEARITY OF CALIBRATION CURVE—

For the high-sensitivity modification, the calibration curve was linear within ±2 per cent. up to at least 5 p.p.m. of silica in the sample; the slope with 4-cm cuvettes at 810 mμ was 0·72 p.p.m. of silica per optical-density unit.

REPRODUCIBILITY—

To measure the reproducibility of the optical-density reading used for calculating the analytical results (*i.e.*, sample *minus* reagent blank optical densities), the procedure described below was used. In one batch, duplicate samples (S_1, S_2) of sodium silicate solution and duplicate reagent blank solutions (B_1, B_2) were treated and measured as in the high-sensitivity modification of the method, and the differences, $S_1 - B_1$ and $S_2 - B_2$, were calculated (4-cm cuvettes were used). The procedure was repeated on each of another 9 days, and the differences were analysed by the usual statistical techniques to obtain estimates of the within- and between-batch standard deviations. Table I shows the results expressed as p.p.m. of silica. The within-batch standard deviation of the reagent blank solution is not directly comparable with the other standard deviations, as it is obtained from single figures and not from the difference, sample less blank. Table I also shows the results for reagent blanks analysed over a longer time period.

TABLE I

REPRODUCIBILITY OF ANALYTICAL RESULTS

Silicon in final solution, p.p.m. of silica	Period of test	Within batch		Between batch	
		Standard deviation, p.p.m. of silica	Degrees of freedom	Standard deviation, p.p.m. of silica	Degrees of freedom
0·47	3.9.60 to 6.10.60	0·0011	10	0·0007	9
0·19	3.9.60 to 6.10.60	0·0005	10	0·0007	9
0·01	3.9.60 to 6.10.60	0·0007	10	n.s.	9
Reagent blank*	3.9.60 to 6.10.60	0·0002	10	—	—
Reagent blank*	12.5.60 to 12.1.62	0·0004†	19	—	—

* Reagent blank figures only and not the difference, sample *minus* blank.

† One extremely bad result excluded.

n.s. = Not significant; variations all assigned to within-batch errors.

EFFECT OF OTHER SUBSTANCES—

Table II shows the effects at 810 mμ of some substances likely to be found in power-station waters.

TABLE II

EFFECT OF OTHER SUBSTANCES

Substance	Silicon in final solution (as silica), p.p.m.	Concentration of substance in final solution at which effect was equivalent to 0·001 p.p.m. of silica, p.p.m.	Effect at higher concentrations of substance, p.p.m. of silica per p.p.m. of substance
Sodium nitrate	0, 0·5	>1000	Not determined
Magnesium chloride	0, 0·5	>1000	Not determined
Calcium sulphate	0	300	Not determined
	0·5	>1000	
Orthophosphate	0, 0·01, 0·2	10 to 25	+0·0000 to 0·0002
Hydrazine sulphate	0, 0·01, 0·2	>10	Not determined
Cyclohexylamine	0, 0·01, 0·2	>10	Not determined
Morpholine	0, 0·01, 0·2	>10	Not determined
Octadecylamine	0, 0·01, 0·2	0·25	+0·0036
Cupric copper	0, 0·01, 0·2	1·7 at 810 and 670 mμ	+0·00056
Ferric iron	0, 0·01, 0·2	>20	Not determined
Ferrous iron in the presence of phosphate	0	0·36 for 0·5 p.p.m. of PO_4^{3-}	about + 0·02
	0	0·27 for 1·0 p.p.m. of PO_4^{3-}	about +0·04
	0	0·07 for 2·0 p.p.m. of PO_4^{3-}	+0·025
	0	0·07 for 5·0 p.p.m. of PO_4^{3-}	+0·025
Ferric hydroxide	0	6	Depends on whether or **not** the ferric hydroxide **is** dissolved by the reagents
Fluorescein	0, 0·5	>10	Not determined

CORRECTNESS OF REAGENT BLANK VALUE—

The correctness of the reagent blank value depends on the assumption that silicon added after the tartaric acid reagent does not form molybdosilicic acid. This assumption was checked by adding 5 ml of the silicon solution containing 10 p.p.m. of silica both with and just after the tartaric acid reagent. In both tests the change in measured silicon content, compared with the normal reagent blank value, was less than 0·001 p.p.m. of silica.

APPLICATIONS TO WATER SAMPLES—

The method was used for analysing de-ionised water from many power-stations. The within-batch standard deviation of the results was 0·0012 p.p.m. of silica (duplicate determinations made on 29 samples) for silicon concentrations ranging from 0·004 to 0·234 p.p.m. of silica. In recovery tests on two samples of power-station de-ionised water and one sample of feed-water, silicon (0·050 p.p.m. of silica) was added to samples and then determined; the maximum discrepancy detected between determined and added amounts was 0·0025 p.p.m. of silica.

DISCUSSION OF THE METHOD

PRECISION AND LIMIT OF DETECTION—

Table I shows that most of the random errors occurred within a batch and that these within-batch errors were equivalent to a coefficient of variation of about ±0·2 per cent. at the 0·5 p.p.m. of silica level. This error is low for an absorptiometric method and probably consists largely of spectrophotometric errors. Most of the important chemical factors were, therefore, probably controlled.

The limit of detection, defined as $1{\cdot}65\sqrt{2}$ times the within-batch standard deviation of the reagent blank,[3] was 0·0008 p.p.m. of silica (95 per cent. confidence level). As the major source of error seems to be the spectrophotometric measurements, this limit may possibly be improved by using 10-cm cuvettes.

APPLICATION TO POWER-STATION WATERS—

Tests at power-stations have indicated that their waters had appreciable absorptions, presumably caused by turbidity and/or coloured materials. Of these two, turbidity seems more important, as we know of no coloured materials (having sufficiently large optical densities) likely to occur in the samples of interest; colour is, therefore, not further considered. As silicon concentrations are often 0·02 p.p.m. of silica or less, optical-density errors as small as 0·003 represent an appreciable bias (about 10 per cent.). The bias caused by turbidity should therefore either be estimated or eliminated by removing the offending materials; a technique for estimating this bias is described under "Procedure," but may not always be valid. The turbidity could probably be reduced to insignificance by filtering the sample through a Millipore filter; tests would be required to determine a suitable pore-size.

Of the substances tested for interference effects only octadecylamine seems likely to be present in some power-station waters in sufficient amounts to cause interference.

SPEED OF ANALYSIS—

The time of analysis is about 1½ hours for a batch of ten samples in duplicate.

EXTENSION TO NATURALLY OCCURRING WATERS—

The method would probably be suitable for analysing naturally occurring waters, but interference effects would have to be checked for each application. For some samples, *e.g.*, sea-water, the effect of large concentrations of salts may be important. This effect has not been studied in detail by us, but approximate estimates indicate that the optical density obtained for a given concentration of silicon is decreased by 1 per cent. for salt concentrations of about 3000 to 4000 p.p.m. in the final solution. In analysing such samples, this difficulty can be overcome (*a*) by using a small aliquot of the sample in order to reduce the salt content of the final solution and (*b*) by preparing the calibration curve with solutions containing the same concentrations of salts as those in the samples; the precision will probably be reduced in this instance.

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Gamma-ray Spectrometric Determination of Low Concentrations of Radioactive Caesium in Sea Water by a Nickel Ferrocyanide Method

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A simple, fairly rapid and reliable method is presented for determining radioactive caesium in sea water, based on a known method of concentration. Radioactive caesium, together with microgram amounts of stable caesium naturally present in sea water, is co-precipitated with nickel ferrocyanide from a 50- to 200-litre sample. After the precipitate has settled and the supernatant liquid has been syphoned off, the nickel ferrocyanide is transferred to a pint container and counted in a gamma-ray spectrometer equipped with a 5-inch × 4-inch NaI(Tl) crystal and 256-channel pulse-height analyser. Studies with a caesium-134 tracer helped to determine the most favourable conditions of pH, temperature and concentration of reagent. The procedure has been used aboard ship and in the laboratory since 1960 for oceanographic survey of radioactive caesium in sea water in concentrations ranging between 0·03 and 0·4 pC per litre.

THE study of the concentration of radioactive caesium in sea water is becoming a subject of great interest. For example, it has been reported recently[1,2] that in human rib bones caesium-137 may sometimes contribute more to the total dose of ionising radiation than in commonly considered to be contributed by strontium-90 in these tissues.

A great deal of information about the distribution of fallout in man's environment is coming from the studies of the distribution of fission product concentrations in the oceans. Caesium-137 is convenient for this purpose because of its long half-life (27 years) and because of the 0·66-MeV gamma ray it emits through its daughter barium-137.

A number of procedures for determining the concentration of caesium in fresh water, fission-product wastes, biological materials and in sea water have been studied. They consist of ion-exchange techniques,[3,4] co-crystallisation as alum[5] and precipitation and co-precipitation as chloroplatinate, perchlorate, cobaltinitrite,[6] dipicrylaminate,[7] ammonium molybdophosphate,[3,8,9] molybdosilicate,[10] tungstophosphate,[11] tetraphenylborate,[3,12,13] cobaltinitrite and tungstosilicate[14] and the ferrocyanides of copper, iron, cobalt and nickel.[15,16,17*] Many investigators have recommended a combination of these techniques to improve the purity and to reduce the size of the caesium sample to be counted. This treatment is essential when the caesium is determined by counting the beta rays. Although several of these methods were reasonably satisfactory, they involved considerable time and delicate chemical processing.

In a search for a quick and simple procedure especially suited for shipboard operations, a procedure, in which is used the co-precipitation of caesium by sodium potassium cobaltinitrite and counting on a gamma-ray spectrometer, has been developed in these laboratories.[18] This procedure, unfortunately, had two difficulties; one was the requirement to keep the temperature below about 19° C and the other was to contend with the significant amount of potassium present, which added to the gamma-ray background. Hence the procedure described below, with nickel ferrocyanide as the concentrating agent (used previously at Hanford Atomic Works to scavenge caesium from fission wastes), was studied as to its usefulness for sea-water analysis.

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[*] We acknowledge a personal communication from Mr. Sadakiyo Hori of the Japan Hydrographic Office, Tokyo (said to have originated from R. Higano of the same institution) proposing a procedure somewhat similar to that described in this paper, but at a time when preliminary tests were already being made here.

EXPERIMENTAL

A known amount of caesium-134 chloride solution was added to 200 litres of sea water, mixed well and distributed into eight 25-litre polythene containers. The influence of the four variables—reagent concentration, pH, temperature and amount of caesium carrier added—was studied. After adjusting the particular variable, solutions of nickel sulphate and potassium ferrocyanide were added, and the contents were stirred for 20 minutes; the reaction appeared to be complete in less than 10 minutes. In all tests the ferrocyanide concentration used was twice the stoicheiometric requirement of the nickel. The precipitate at first is a milky opalescence, gradually increasing in particle size and finally settling down as a distinct layer of amorphous light green sludge; the supernatant liquid is clear and colourless. After the precipitate had been allowed to settle for 24 hours, the supernatant liquid was syphoned off. The precipitate was collected in a commercially available cylindrical polythene container, 10 cm in diameter and 8 cm high. After the precipitate had been allowed to settle for another day, its height was measured and recorded for applying geometrical correction; the precipitate was then counted in a gamma-ray spectrometer, the container being placed on top of a sodium iodide crystal 5 inches in diameter and 4 inches thick.[18]

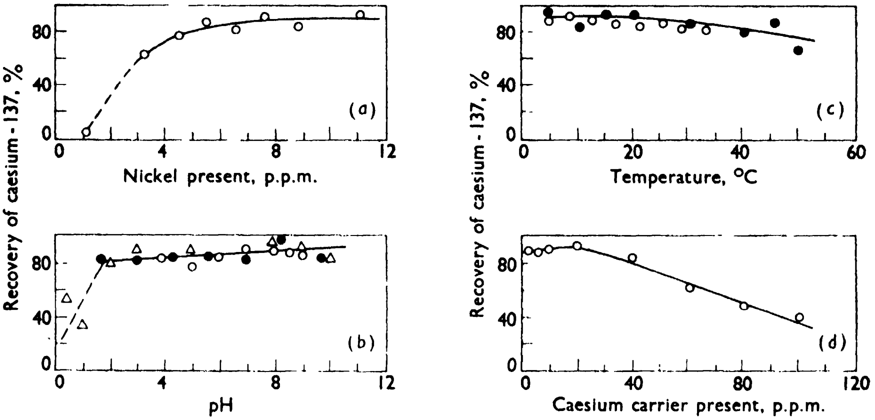

Fig. 1. Influence of four variables on the recovery of caesium-137

(*a*) Influence of reagent concentration; temperature 24° C; pH 8·3

(*b*) Influence of pH: ○, 22° C, nickel concentration 6·8 p.p.m.; △, 19·5° C, nickel concentration 11·0 p.p.m.; ●, 20° C, nickel concentration 11·0 p.p.m.

(*c*) Influence of temperature: ○, pH 8·0, nickel concentration 11·0 p.p.m.; ●, pH 8·1, nickel concentration 6·8 p.p.m.

(*d*) Influence of caesium carrier; temperature 22° C; pH 8·4; nickel concentration 11·0 p.p.m.

RESULTS

The influence of the four variables on the recovery of radioactive caesium in sea water is shown in Fig. 1. The recovery of caesium increased with increasing concentrations of nickel up to 7 p.p.m., becoming essentially constant between 7 and 12 p.p.m. (see Fig. 1(*a*)). From this it was concluded that a nickel concentration of 11 p.p.m. was adequate, and this concentration was used for most of the experiments. Results from three independent experiments are shown in Fig. 1(*b*), which indicate that the caesium recovery decreases slightly with decreasing pH down to 2; recovery was unreliable and low below pH 2. This behaviour makes the procedure especially suitable for use with average sea water, which has a pH of 8·2.

Fig. 1(*c*), which shows results of two experiments, indicates that the recovery of caesium decreases slightly with increase in temperature up to 30° C and somewhat more significantly above 30° C. It can be seen from Fig. 1(*d*), which shows a study of the effect of variations in the amount of carrier used, that the recovery of caesium-137 was not improved to any significant extent by the addition of caesium chloride carrier up to 20 p.p.m. The recovery was reduced with increase in the amount of carrier above 20 p.p.m.; apparently this was because insufficient nickel ferrocyanide precipitate was found.

The recovery and reproducibility of the method was studied by precipitating and counting 8 labelled replicate samples of fresh sea water under identical conditions. Three such experiments were conducted under slightly different conditions. The precipitates from one experiment were counted in a 3-inch × 3-inch sodium iodide well crystal; the other two were counted on a 5-inch × 4-inch solid crystal. Results from the three sets of experiments are shown in Table I and indicate that the average recovery was between 89 and 90 per cent.; the coefficient of variation of measurements in each experiment lay between $\pm$3·1 and $\pm$5·6 per cent. of the average recovery.

TABLE I

RECOVERY OF CAESIUM-137 FROM LABELLED REPLICATE SAMPLES OF SEA WATER

Replicate No.	Recovery of caesium-137 counted with—		
	5-inch × 4-inch flat crystal		well crystal
	A,* %	B,* %	C,* %
1	85·0	84·0	91·1
2	97·5	84·0	88·0
3	82·3	88·6	94·5
4	84·4	91·1	90·7
5	92·0	91·1	87·4
6	93·0	88·6	86·3
7	96·4	88·6	86·1
8	89·6	95·9	89·0
Average	90·0	89·0	89·1
Standard deviation ..	5·0	3·9	2·8
Coefficient of variation ..	5·6	4·4	3·1

* Solution conditions were—
A: 6·8 p.p.m. of Ni^{2+}; pH 8·2; temperature 19° C.
B: 11 p.p.m. of Ni^{2+}; pH 8·1; temperature 17° C.
C: 11 p.p.m. of Ni^{2+}; pH 8·2; temperature 22° C.

TABLE II

DETERMINATION OF CAESIUM-137 IN SEA WATER

Sample size = 200 litres.
Detector = 5" × 4" NaI crystal.
Time of counting = 8 hours.
Spectrometer = 256 channels (set at 0 to 1·6 MeV)

pH = 8·2 (natural).
Temperature = 15° C.
Fe(CN)$_6^{4-}$ = 40 p.p.m. (added)
Reagent blank value = 0·015 pC per litre.

Replicate No.	1	2	3	4	5	Mean	Standard deviation
Caesium-137 found, pC per litre	0·133	0·151	0·155	0·151	0·143	0·147	0·01

Coefficient of variation* = 6·8%

* The coefficient of variation of about 7 per cent. might appear fortuitous. However, a graphical smoothing has been carried out on the spectral record so that information equivalent to nine times that in the peak channel was used. Because of this, the coefficient of variation of measurements of this type and concentration should be about 4 per cent. The experimental value is therefore not unreasonable.

TEST WITH UNLABELLED SEA WATER—

The procedure was applied to five 200-litre replicate samples of sea water collected at Scripps pier. Table II shows that the sea water contained 0·147 $\pm$ 0·01 pC of caesium-137 per litre. The reagent blank values were no more than about 0·015 pC per litre. Many samples have been collected from the Pacific Ocean since 1960 and analysed by this method; the results are reported elsewhere.[19,20]

STIRRING REQUIREMENTS—

Stirring requirements were determined as described below. Four labelled 10-litre samples were each stirred, after the reagent had been added, by an 1800 r.p.m. stirrer having a 3-cm × 3-cm blade for 5, 20, 60 and 60 minutes; the precipitates were then allowed to

settle before re-stirring for a further 60 minutes. The recoveries of caesium were 90, 87, 84 and 93 per cent., respectively. From this it would appear that the previously used stirring time of 20 minutes has been adequate. Presumably equilibrium is reached rapidly because of the large area provided by the fineness of the solid suspension.

CONCLUSIONS

Determination of caesium in sea water by co-precipitation with nickel ferrocyanide has been found to be feasible. Neither adjustment of pH or temperature nor addition of carrier was found necessary. When counted for 8 hours on a 5-inch × 4-inch sodium iodide crystal, 200-litre samples gave results with a standard deviation of ± 7 per cent. of the mean. The simplicity of the method makes it convenient for processing samples on board the ship so that precipitates (in pint containers) can be sent home in place of 55-gallon samples of unprocessed sea water. Unlike the cobaltinitrite method previously used in this laboratory, the proposed method is useful even at tropical temperatures.

The method, unfortunately, requires one day or more for the precipitate to settle in the 55-gallon drum, and two or more days in smaller containers. Studies now being undertaken at these laboratories indicate that the rate of settling can be remarkably increased by application of available coagulants, and practical methods will be reported separately.

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The Colorimetric Determination of Aluminium in Minerals by Pyrocatechol Violet

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A procedure is described for the colorimetric determination of aluminium in small mineral samples with pyrocatechol violet. Conditions for the formation of this complex and the effects of other cations and anions have been examined. The optimum pH in acetate buffer solution is 6·1 to 6·2. Interference from iron is considerably reduced by the formation of the ferrous - *o*-phenanthroline complex.

The determination of microgram amounts of aluminium, as for example in the microanalysis of minerals, is made difficult by the lack of a satisfactory colorimetric reagent for aluminium. The available reagents, such as alizarin, aluminon and Eriochrome cyanine R, are generally characterised by low specificity and a tendency to give high blank values because of their high optical densities at the optimum wavelength. Probably the best of the existing methods is the formation of the oxinate and extraction of this with chloroform to give a yellow-coloured solution of the complex. This method is applicable to many of the common silicate rocks in which there are likely to be few interferences, and where interference due to iron in particular can be almost eliminated by complex formation with *o*-phenanthroline.

The reactions of pyrocatechol violet with a number of metals in solution have been examined by Suk and Malat,[1] who found that the stability of the blue and yellow complexes formed increased with the ionic charge of the metal ion complexed. In slightly acid solutions, bivalent ions did not form complexes, so that it might be expected that, in addition to the alkaline earths, such metals as manganese, beryllium and uranium, in the uranyl form, would not cause interference. The colour reaction with aluminium is distinctive, as the complex is blue in strong contrast to the yellow colour of pyrocatechol violet in slightly acid solution.

A method for the colorimetric determination of aluminium by pyrocatechol violet has recently been described by Anton,[2] and it was decided to investigate the reaction further with particular reference to the micro-determination of aluminium in minerals also containing manganese, uranium, beryllium and the rare earths.

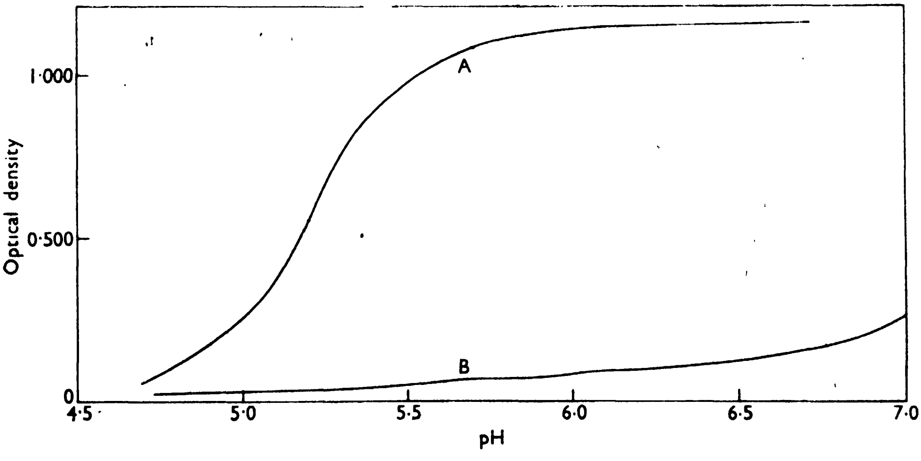

Fig. 1. Variation in optical density of the aluminium - pyrocatechol violet complex with pH. Optical densities measured in 1-cm cells against water after 2 hours: curve A, solutions containing 80 µg of aluminium oxide, 1 ml of 0·2 per cent. pyrocatechol violet solution, 50 ml of 10 per cent. ammonium acetate buffer solution and 2 ml of 10 per cent. hydroxylamine hydrochloride solution per 100 ml; curve B, reagents only

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EXPERIMENTAL

pH AND COMPLEX FORMATION—

Anton formed the aluminium - pyrocatechol violet complex at pH 5·0 with a pyridine acetate buffer. We have found that the maximum colour is developed at a higher pH, as shown in Fig. 1, from which it can also be seen that above pH 5·9 the optical density rises to a fairly constant level. The optical density of the reagent also increases with pH, at first slowly and then, above pH 6·4, much more rapidly as the colour turns blue. The optimum pH for colorimetry of the aluminium complex lies between 5·9 and 6·4, and a satisfactory working range is pH 6·1 to 6·2 with ammonium acetate - acetic acid buffer. It becomes necessary in this pH region, which is difficult to stabilise, to adjust the pH to nearly the required value before adding the buffer solution.

WAVELENGTH AND OPTICAL DENSITY—

The variation in optical density with wavelength of both pyrocatechol violet and the aluminium complex was studied at pH 6·1 to 6·2; the results are shown in Fig. 2, from which it can be seen that there is a maximum for the complex at 580 mμ, and this wavelength was chosen for subsequent measurements.

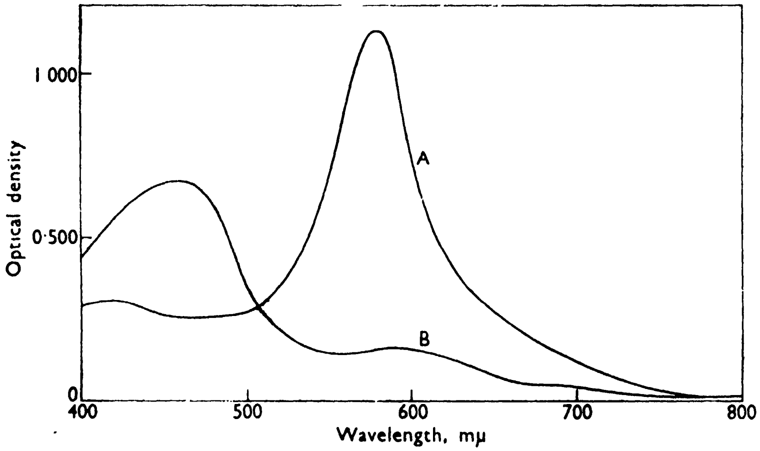

Fig. 2. Variation in optical density with wavelength. Optical densities measured in 1-cm cells against water after 2 hours; curve A, solutions containing 80 μg of aluminium oxide, 1 ml of 0·2 per cent. pyrocatechol violet solution, 50 ml of 10 per cent. ammonium acetate buffer solution and 2 ml of 10 per cent. hydroxylamine hydrochloride in a total volume of 100 ml (pH 6·2); curve B, reagents only

TIME OF DEVELOPMENT OF COLOUR—

Under the conditions chosen for the reaction, maximum colour development was found to take place in approximately 1 hour, after which the colour intensity remained virtually constant for several hours.

BEER'S LAW AND REAGENT CONCENTRATION—

For a range of concentrations up to 80 μg of aluminium oxide per 100 ml, the colour intensity follows Beer's law provided that a sufficient excess of reagent is present (2 ml of 0·15 per cent. w/v aqueous solution).

INTERFERENCES AND APPLICATIONS—

The determination of aluminium in calcium-bearing minerals presents no difficulties, but, when elements such as iron, beryllium, chromium, etc. (see Table I) are present, two factors must be considered; (*a*) the precision required of the method and (*b*) the ratio of aluminium to the interfering metal. Then, if necessary, a correction can be applied or the interfering elements can be removed. For example, a number of strongly interfering cations, such as thorium, zirconium and vanadium but not chromium, are simply removed, together with iron and titanium, by an acid cupferron extraction. In the presence of hydroxylamine and *o*-phenanthroline, interference by iron is greatly diminished by the formation of the ferrous - *o*-phenanthroline complex, which absorbs only slightly at 580 mμ. A routine addition of 5 ml of 0·15 per cent. w/v aqueous *o*-phenanthroline is sufficient when the ratio of ferric oxide to alumina does not exceed 4 to 1. The method could be satisfactorily applied in the analysis of an average silicate rock in which the only element likely to be present in sufficient amount to interfere would be titanium, for which a correction could be applied.

Both phosphate and fluoride might be expected to interfere as these anions tend to form stable complexes with aluminium. The effect of fluoride is especially important, as fluoride is likely to be present after the decomposition of silicates by mixed hydrofluoric and sulphuric or perchloric acids. The results in Table I show that 50 μg of fluoride per 80 μg of alumina can be tolerated; this is much more than would normally remain as residual fluoride after the usual mixed acids treatment. Phosphate occurs in small concentrations in many silicate rocks and minerals and may be a major constituent, especially of calcium and uranium minerals. Fifty micrograms of phosphate (as P_2O_5) per 80 μg of alumina were found not to interfere significantly. Larger amounts of phosphate tend to suppress the colour, and in the analysis of phosphatic material it would be necessary to add an equivalent amount of phosphate to the standards, a modification that can also be applied to compensate for other interfering radicals.

TABLE I

INTERFERENCE OF VARIOUS IONS

Element added	Ionic species added	Concentration, μg per 100 ml	Optical density	Interference, %
Aluminium ..	Al^{3+}	80 as Al_2O_3	1·060	—
Iron	Fe^{3+}	1000 as Fe_2O_3	0·086	+8·1
		50 as Fe_2O_3	0·004	+0·4
Uranium	UO_2^{2+}	1000 as UO_3	0·014	+1·3
Beryllium	Be^{2+}	1000 as BeO	0·078	+7·4
		50 as BeO	0·004	+0·4
Manganese	Mn^{2+}	1000 as MnO	0·034	+3·2
		50 as MnO	0·002	+0·2
Chromium	CrO_4^{2}	50 as Cr_2O_3	0·109	+10·3
Vanadium	VO_3^-	50 as V_2O_5	0·046	+4·3
Titanium	Ti^{4+}	50 as TiO_2	0·188	+17·8
Calcium	Ca^{2+}	5000 as CaO	0·002	+0·2
Magnesium ..	Mg^{2+}	5000 as MgO	0·002	+0·2
Lanthanum ..	La^{3+}	5000 as La_2O_3	0·080	+7·5
Cerium	Ce^{3+}	5000 as Ce_2O_3	0·050	+4·7
Fluoride	F^-	200 as F	1·022	−3·6
Aluminium ..	Al^{3+}	80 as Al_2O_3		
Fluoride	F^-	50 as F	1·060	0·0
Aluminium ..	Al^{3+}	80 as Al_2O_3		
Phosphorus ..	PO_4^{3}	200 as P_2O_5	1·035	−2·5
Aluminium ..	Al^{3+}	80 as Al_2O_3		
Phosphorus ..	PO_4^{3}	50 as P_2O_5	1·059	−0·1
Aluminium ..	Al^{3+}	80 as Al_2O_3		

METHOD

REAGENTS—

Pyrocatechol violet solution, 0·15 per cent. w/v, aqueous.

Hydroxylamine hydrochloride solution, 10 per cent. w/v, aqueous.

o-Phenanthroline solution, 0·15 per cent. w/v, aqueous.

Buffer solution—Prepare a 10 per cent. w/v aqueous solution of ammonium acetate, and adjust the pH to 6·2 with acetic acid (approximately 0·4 per cent. w/v of acetic acid is required).

Cupferron solution, 1 per cent. in chloroform—Prepare by acidifying an aqueous solution of cupferron and extracting into chloroform.

PROCEDURE—

To approximately 20 ml of slightly acid test solution containing not more than 40 μg of aluminium, add 2 ml each of hydroxylamine hydrochloride solution, *o*-phenanthroline solution and pyrocatechol violet solution and then 5 ml of buffer solution. Mix well, and adjust to pH 6·1 to 6·2 by careful additions of dilute ammonia solution and dilute acetic acid, care being taken not to allow the solution at any time to become distinctly alkaline. Transfer the solution to a 100-ml calibrated flask, and add a further 50 ml of buffer solution as rinsings. Dilute to the mark, mix the solution, set aside for 2 hours, and measure the optical density against distilled water in 1-cm cells at 580 mμ. Apply a correction for the reagent blank value.

EXTRACTION PROCEDURE—

When necessary, precede the colour development by a cupferron extraction.

Add 2 ml of 12 N hydrochloric acid to about 20 ml of solution contained in a small separating funnel. Extract interfering elements with successive 5-ml portions of a 1 per cent. solution of cupferron in chloroform. Wash the solution twice with chloroform and once with light petroleum. Continue with the procedure described, omitting the addition of *o*-phenanthroline.

TABLE II

COMPARISON OF RESULTS

Solutions were prepared from 1·000-g samples of rock powder; portions containing between 300 and 500 μg were used in the determinations

Sample laboratory No.	Interfering elements		Alumina found by	
	Total ferric oxide, %	Titanium dioxide, %	gravimetric method, %	proposed method, %
1543	11·2	1·1	15·0	15·1, 14·9
1771	1·3	0·06	12·2	12·8, 12·9
1821	17·1	2·0	21·3	21·4, 21·8
1822	19·6	2·2	23·8	23·8, 24·8
1823	15·9	4·9	19·6	19·9, 19·3
1836	11·3	1·3	15·1	15·0, 15·0
1816*	12·7	1·3	18·2	18·1, 18·6, 18·0, 17·7

* Re-ground sample.

RESULTS

The proposed method is intended for the rapid evaluation of aluminium in milligram samples of minerals. However, in order to test the procedure, the method was applied to a selection of previously analysed silicate rocks from the Geological Survey collection. Since rock specimens, in contrast to mineral specimens, are not homogeneous, large amounts were taken for the purpose of this test to avoid sampling errors; the necessary concentrations for the procedure were obtained by suitable dilution. Corrections were applied for iron and titanium. The results are shown in Table II; the reproducibility is only moderate but is sufficiently good for the intended applications. In obtaining these results, no analytical separations, other than the removal of silica, were made.

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The Determination of Scandium in Uranium Compounds by Solvent Extraction with 2-Thenoyltrifluoroacetone

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A method is described for determining scandium in uranium compounds. In the proposed procedure scandium is separated from interfering elements by extraction into a 0·2 M solution of 2-thenoyltrifluoroacetone in xylene. The scandium is then separated from the organic phase with nitric acid and determined spectrophotometrically as the red-brown coloured scandium - alizarin complex. Of the elements usually found in uranium compounds only copper interferes.

EXISTING methods for determining scandium in the microgram range, especially in the presence of thorium, are time-consuming and not well suited to routine analysis. A method suitable for determining scandium in uranium compounds on a routine basis was not available. Volumetric and gravimetric methods were too insensitive, and solvent extraction appeared to offer the best approach to the problem.

N-Benzoylphenylhydroxylamine has been used for separating scandium,[1] but in the presence of thorium, uranium and other elements no ready separation of scandium could be achieved. Broido[2] has used 2-thenoyltrifluoroacetone (TTA) for extracting scandium for separation and purification purposes. Preliminary work with this reagent showed promise, and has resulted in the development of a rapid and accurate method for determining scandium in the microgram range in uranium compounds.

EXPERIMENTAL

The use of alizarin sulphonic acid as the chromotropic reagent for the spectrophotometric determination of scandium was found to provide the required sensitivity, and this reagent has been used throughout this work. The scandium - alizarin complex was developed in an acetate - acetic acid buffer at pH 3·5. Beer's law was obeyed over the range 0 to 200 µg of scandium in a volume of 100 ml.

EXTRACTION OF SCANDIUM—

Scandium was extracted from solutions adjusted to different pH values in the range 1 to 6 by shaking for 3 minutes with a 0·2 M TTA - xylene solution, and separated from the organic phase with 2 M nitric acid. The scandium was then determined with alizarin. Quantitative extraction was given over the pH range 2·5 to 6 (see Fig. 1).

At about pH 2 and above, uranium precipitates from solution, especially when amounts of the order of 1 g in 50 to 100 ml are present. In order to keep the uranium in solution above pH 2 several complexing agents were tried. Tartaric acid was satisfactory for the purpose, but small amounts were found to bleach the colour of the scandium - alizarin complex. Consequently it was necessary to remove all traces of tartaric acid from the organic phase before separating the scandium. Washing the organic phase with water tended to produce emulsions, and the presence of an electrolyte was found necessary to inhibit emulsion formation. A 10 per cent. w/v solution of sodium nitrate was found to be suitable for this purpose.

In the presence of tartaric acid, the pH range for complete extraction of scandium was decreased from 2·5 to 6 to 5 to 6 (see Fig. 1).

INTERFERING ELEMENTS—

Elements found to give a colour with alizarin at pH 3·5 were iron^{III}, vanadium^{V}, thorium, aluminium, copper^{II}, zirconium, titanium^{III}, titanium^{IV}, molybdenum, tin^{II} and uranium^{VI}. Elements that did not give a colour and consequently did not interfere were lead, bismuth, chromium^{VI}, zinc, cobalt, arsenic^{V}, antimony^{V}, magnesium, calcium, nickel, sodium and potassium. The amount of each element added was 10 mg. Of the elements giving a colour with alizarin, thorium, vanadium^{V}, titanium^{III}, titanium^{IV} and copper^{II} were found to interfere after extraction with TTA - xylene solution.

Thorium—Thorium is extracted into TTA - xylene solution above about pH 0·5 (see Fig. 1). In the presence of tartaric acid, the range for quantitative extraction is pH 3 to 4 (see Fig. 1). At pH 5, about 50 per cent. is extracted, and at pH 6 the amount extracted drops to about 25 per cent. These results were given when 2 M nitric acid was used to separate the thorium from the TTA - xylene solution.

In the absence of tartaric acid, thorium is quantitatively extracted into TTA - xylene solution at pH 1 (see Fig. 1) and will not, therefore, be separated from the organic phase with nitric acid at pH 1. Scandium is extracted into TTA - xylene solution to the extent of about 20 per cent. from solutions at pH 1, and can be separated from the organic phase with nitric acid solution at pH 2.

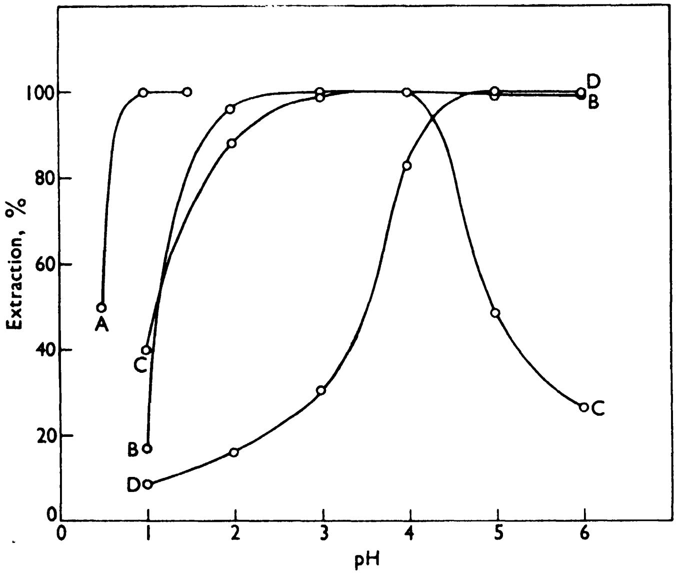

Fig. 1. Extraction of scandium and thorium: curve A, thorium (no tartaric acid); curve B, scandium (no tartaric acid); curve C, thorium in presence of tartaric acid; curve D, scandium in presence of tartaric acid

A solution containing 67 μg of scandium and 100 μg of thorium, adjusted to pH 5·5, was shaken with 0·2 M TTA - xylene solution. After washing the organic phase with tartaric acid solution adjusted to pH 5 with ammonium hydroxide, and then with a 10 per cent. solution of sodium nitrate, the scandium was separated from the organic phase with nitric acid solution at pH 1. Complete extraction of scandium was given, with no interference from thorium. Tests carried out with up to 1560 μg of thorium and 67 μg of scandium showed that less than 20 μg of thorium was extracted by the procedure described above; thoron was used to determine the thorium—

Thorium added, μg	260	520	1040	1560
Thorium extracted, μg	10	7	18	14

As shown in Table I, 20 μg of thorium gives a colour with alizarin having an optical density equivalent to 2 μg of scandium.

Vanadium and titanium—Addition of hydrogen peroxide to the sample solution before extraction eliminated interference from both vanadium^{V} and titanium^{III} and titanium^{IV}. Addition of hydrogen peroxide below pH 2 to 3 caused precipitation of uranium, presumably as UO_4; addition at pH 4 to 5 was satisfactory.

Copper—Amounts of copper greater than 2 mg caused precipitation of a copper - alizarin complex at pH 3·5. Only extremely small amounts of copper (<50 μg) can be tolerated.

Extraction with dithizone, or sulphide precipitation if sufficient copper is present, may be used to remove the copper.

Interfering anions—Fluoride and phosphate interfere in the procedure and must be absent. Up to 1 g each of sodium sulphate and sodium chloride do not interfere.

TABLE I

EFFECT OF THORIUM ON THE DETERMINATION OF SCANDIUM

Thorium added, µg	Optical density at 530 mµ	Scandium equivalent to optical density, µg
5	0·001	1
10	0·001	1
20	0·002	2
50	0·010	12
100	0·015	19
1000	0·125	158

METHOD

REAGENTS—

2-Thenoyltrifluoroacetone, 0·2 M—Dissolve 25 g of 2-thenoyltrifluoroacetone in 500 ml of xylene.

Tartaric acid, 20 *per cent. w/v.*

Tartaric acid wash solution, 5 *per cent.*—Adjust to pH 5 with ammonium hydroxide.

Sodium nitrate, 10 *per cent. w/v.*

Nitric acid, pH 1·0—Adjust about 1 litre of water to pH 1·0 with nitric acid.

Hydrogen peroxide, 30 *per cent.*

Buffer solution—Adjust a 10 per cent. w/v solution of sodium acetate to pH 3·5 with acetic acid.

Alizarin sulphonic acid, 0·05 *per cent., aqueous.*

PROCEDURE—

For solid samples, weigh a suitable portion into a platinum crucible, add about 5 g of peroxide, and mix thoroughly; sinter at 400° to 450° C for 1 hour. Cool, dissolve the melt in water, just acidify with nitric acid, and boil for several minutes. Cool the solution, and dilute to a suitable volume.

Transfer a portion of solution containing up to 200 µg of scandium to a 100-ml beaker. Add 10 ml of 20 per cent. w/v tartaric acid solution, and, with use of a pH meter, adjust the pH to about 5 with ammonium hydroxide. If necessary, cool the solution to room temperature. Add 2 ml of 30 per cent. hydrogen peroxide, and adjust the pH to 5 to 5·5. Transfer the solution to a 125-ml separating funnel, add 20 ml of 0·2 M TTA - xylene solution, and shake vigorously for 3 minutes. Allow the phases to separate, and discard the aqueous phase. Shake the organic phase with 10-ml portions of the tartaric acid wash solution until the aqueous phase is colourless, and discard the washings. Shake the organic phase twice with 10-ml portions of 10 per cent. sodium nitrate solution, shaking for 1 minute at each extraction. Discard the aqueous phase. Separate the scandium from the organic phase twice with 10-ml portions of nitric acid solution at pH 1·0, and run the aqueous phase into a 100-ml calibrated flask. By pipette, transfer 5 ml of the alizarin solution to the flask. Add dilute ammonium hydroxide to the solution until the colour of the solution just changes from yellow to red. Add by pipette 5 ml of the buffer solution, dilute to the mark with water, and mix. Measure the optical density of the solution in 1-cm cells at 530 mµ, the spectrophotometer having been set to zero against a blank solution. Calculate the amount of scandium in the sample from a previously prepared calibration graph.

PREPARATION OF CALIBRATION GRAPH—

Dissolve 0·1534 g of pure scandium oxide (99·9 per cent.) in dilute hydrochloric acid, and dilute to 1 litre. Dilute this solution five-fold to give a solution containing 20 µg of scandium per ml.

Measure from a burette 0-, 1-, 3-, 5-, 7- and 10-ml portions of the scandium solution into 100-ml beakers and continue as described under "Procedure." Plot a graph of optical density against µg of scandium.

TABLE II

DETERMINATION OF SCANDIUM IN THE PRESENCE OF THORIUM AND URANIUM

The solutions contained 0·5 g of sodium diuranate

Scandium added, µg	Thorium added, µg	Scandium recovered, %
65	500	100
65	1000	101
130	500	102
130	1000	105
195	500	99
195	1000	102
260	500	102
260	1000	100

TABLE III

COMPARISON OF PROPOSED METHOD WITH NEUTRON-ACTIVATION ANALYSIS

	Amount of scandium found by—	
Sample	proposed method, p.p.m.	neutron activation, p.p.m.
A	265, 270	257
B	262, 258	258
C	80, 82	—

RESULTS AND CONCLUSIONS

Known amounts of scandium and thorium were added to solutions of sodium diuranate. The results shown in Table II indicate that the recovery of scandium was good. In Table III are shown results obtained on uranium compounds, together with results by neutron-activation analysis.

The procedure has been found suitable for determining small amounts of scandium in uranium compounds and also in lime samples. Dilution to a final volume other than 100 ml may be used; for example, if traces of scandium are to be determined, a final dilution of 50 ml or the use of 5-cm cells will increase the sensitivity of the procedure.

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The Quantitative Micro-determination of Biphenyl in Citrus Fruit

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A simple and rapid colorimetric method is described for the micro-determination of biphenyl in citrus fruit; it is based on the specific colour reaction given by biphenyl with sulphuric acid, traces of formaldehyde and ferric iron. A sample of the ground fruit is steam-distilled, the biphenyl in the distillate is extracted with chloroform, and the extract is purified with 95·2 per cent. w/w sulphuric acid. The biphenyl is then determined colorimetrically. The method is suitable for routine use and permits the determination of small amounts of biphenyl of the order of 0·06, 1·0 and 0·3 p.p.m. in the fruit pulp, peel and whole fruit, respectively; the accuracy is within the limits of experimental error.

BIPHENYL is used as a fungistat for protecting citrus fruit from certain diseases that develop during storage and transport. In the past few years several countries have set limits for biphenyl residues in citrus fruit, and thus it became necessary to be able to discern untreated fruit and to determine minute amounts of biphenyl in treated fruit.

The various colorimetric methods[1,2,3] described are rather laborious, and the accuracy of spectrophotometric methods[4,5,6,7,8]—despite the great efforts made to eliminate interfering substances[8]—is limited by a variable background or apparent biphenyl value. Further, these methods usually require costly apparatus that is not available in most laboratories.

The method proposed here is both simple and rapid. It is based on a blue colour reaction given by biphenyl with sulphuric acid, traces of formaldehyde and ferric iron.[9] The reaction is specific and sensitive; it makes possible visual identification or quantitative determination of extremely small amounts of biphenyl in fruit. The proposed method is suitable for routine use.

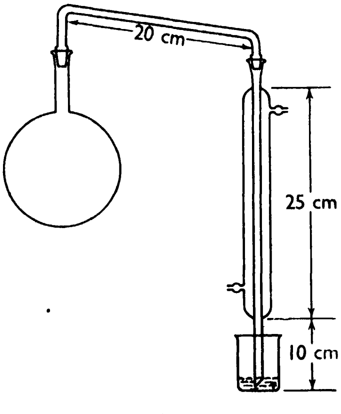

Fig. 1. Distillation apparatus

METHOD

GENERAL DESCRIPTION—

Biphenyl is steam-distilled from a sample of ground fruit and extracted from the distillate with chloroform; the extract is then treated with sulphuric acid to eliminate interfering substances. One volume of purified extract is mixed with nine volumes of acetic acid containing formaldehyde; sulphuric acid containing ferric iron is then added. The optical density of the blue colour developed is measured at 610 mμ. The colour reaction and the determination of biphenyl in chloroform solution have been described in detail in a previous paper.[9]

APPARATUS—

Electric blender.

Shaking machine.

Absorptiometer—A Hilger absorptiometer type H810, with 6-inch × ⅝-inch tubes, was used.

Distillation apparatus—A round-bottomed 2-litre flask, condenser and connection tube, with ground-glass joints (see Fig. 1).

Beakers, 250 ml.

Separating funnels, 100 and 200 ml.

Calibrated flasks, 50 ml, with ground-glass stoppers.

Erlenmeyer flasks, 100 ml.

Graduated tubes—Tubes, 10 ml, graduated at 0·1-ml intervals, with ground-glass stoppers and adaptor for concentration (see Fig. 2).

Test-tubes, 150 mm × 16 mm.

Pipettes, 1, 5 and 10 ml.

Micropipettes—Curved tip 0·1- and 0·2-ml pipettes, graduated at 0·001-ml intervals.

Filter paper, Whatman No. 42.

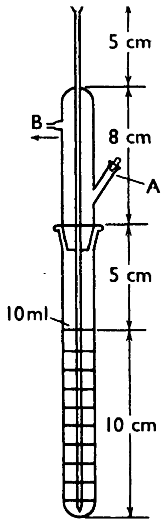

Fig. 2. Graduated 10-ml tube with adaptor for concentration of extract: A, inlet; B, connection to vacuum line

REAGENTS—

All reagents should be of recognised analytical grade.

Standard biphenyl solution—Dissolve 80 mg of pure biphenyl in chloroform, and make up to 100 ml.

Acetic acid reagent—Mix 1 ml of 37 per cent. formaldehyde solution with glacial acetic acid to make 100 ml. Determine colorimetrically the amount of formaldehyde impurity present in the glacial acetic acid, and then add the necessary amount of the prepared solution to more acetic acid to obtain a reagent containing 16·6 mg of formaldehyde per litre. Check the formaldehyde content of the reagent as described below.

Prepare a standard solution containing 16·6 mg of formaldehyde per litre of water by mixing 1 ml of 37 per cent. formaldehyde with distilled water to make 100 ml; then dilute a 4·5-ml portion of this solution to 1 litre. Place 0·0, 0·05, 0·10 and 0·15 ml of the standard solution and 0·05, 0·10 and 0·15 ml of acetic acid reagent in separate test-tubes; add 5 ml of chromotropic acid reagent—500 mg of chromotropic acid in 100 ml of diluted sulphuric acid (4 + 2·8)—to the contents of each test-tube. Place the test-tubes in boiling water for

15 minutes, and then cool, and measure the optical densities at 610 mμ. Set the absorptiometer to zero against the test-tube containing no standard formaldehyde solution. Equal amounts of acetic acid reagent and standard solution should show the same optical densities.

Sulphuric acid reagent—Prepare a stock solution by dissolving 50 g of ferric sulphate in 950 ml of diluted sulphuric acid (1 + 8·5). For use, mix 5 ml of stock solution with 95 ml of 98 per cent. w/w sulphuric acid; prepare daily.

Sulphuric acid, 95·2 per cent. w/w—Dilute 98 per cent. w/w sulphuric acid (1 + 0·053).

Chloroform (stabilised by ethanol).

Bone charcoal powder (not adsorbing biphenyl).

PREPARATION OF STANDARD GRAPH—

Mix 1 ml of standard biphenyl solution with 9 ml of acetic acid reagent in a 10-ml graduated tube. (Each 0·1 ml of mixture contains 8 μg of biphenyl.) Place in separate test-tubes 0·05-, 0·10-, 0·15-, 0·20-, 0·25- and 0·30-ml portions of this biphenyl mixture. Gradually add 5 ml of sulphuric acid reagent to the contents of each test-tube. When a small amount of sulphuric acid reagent has been added, mix vigorously until full colour intensity is obtained; then add the rest of the reagent with thorough mixing. The colour obtained should be clear; any secondary colour, such as grey or violet, indicates failure of the test. Wait a few minutes until gas bubbles have disappeared, and then measure the optical densities at 610 mμ. Set the absorptiometer to zero against the sulphuric acid reagent. Beer - Lambert's law is obeyed up to 14 μg of biphenyl in 5 ml of reagent. The standard graph is reproducible and is valid for solutions containing between 25 and 800 μg of biphenyl per ml of chloroform. Other solutions have to be suitably concentrated or diluted with chloroform.

PRELIMINARY PROCEDURE—

Biphenyl is determined in the peel and in the pulp of fruit and calculated for the whole fruit.

Sampling—From an average sample of fruit, prepare 125 g of peel and about 600 g of pulp. Weigh, for example, 10 fruits, and remove their peels carefully to avoid contaminating the pulp with traces of biphenyl from the peel; put aside from each fruit an average sample of 12·5 g of the peel, making altogether 125 g. Weigh the pulps, and calculate the weight of the total peels. If the pulps weight more than 600 g, take from each pulp an aliquot part.

Preparation of peel sample—Chop the peels on a plate. Place the chopped peels in the electric blender, rinse the plate with 400 ml of distilled water, add the rinsing to the peels, and grind to an impalpable purée; this is essential for the complete extraction of biphenyl. Transfer the purée quantitatively to the distillation flask, rinse the blender with 400 ml of distilled water, and add the rinsing to the purée.

Preparation of pulp sample—Grind the chopped pulp in the electric blender. Transfer the resulting purée quantitatively to the distillation flask, rinse the blender with 200 ml of distilled water, and add the rinsing to the purée.

Distillation—Connect the distillation flask to the condenser (see Fig. 1), which dips into a 250-ml beaker containing 20 ml of distilled water. Heat the flask with a gas burner, and distil. To ensure a clear distillate, boil gently, and avoid production of foam. Collect the first 100 ml of distillate. Use 10 ml of chloroform to rinse carefully the inner tube and the tip of the condenser, and collect the rinsings in the distillate.

Extraction—Transfer the distillate to a 200-ml separating funnel. Rinse the beaker with 5 ml of chloroform, add the rinsings to the contents of the funnel, and shake for 2 to 3 minutes. Carefully decant off the chloroform layer, and extract three more times with 10-ml portions of chloroform. Collect the chloroform layers in a 50-ml calibrated flask, make up to the mark with chloroform, and insert the stopper tightly in the flask.* (These extracts can, if necessary, be stored for some time in the dark.)

Clean-up of interfering substances—Place about 35 ml of the chloroform extract into a 100-ml separating funnel. Add 10 ml of 95·2 per cent. w/w sulphuric acid, and shake for 10 minutes. Decant off the acid layer carefully, and repeat the operation several times. Transfer the purified extract (see "Preliminary Determination") to a 100-ml Erlenmeyer flask, add a little bone charcoal powder, mix, and filter through Whatman No. 42 filter-paper. Collect the filtrate in a 50-ml graduated cylinder, and close carefully. (Record the exact volume to allow correction for eventual evaporation of chloroform.)

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* Avoid all changes in the concentration of the extract. Biphenyl will be determined per ml of extract and calculated per 50 ml.

Preliminary determination—Certain extracts are sufficiently pure for determination, even though the chloroform layer and sulphuric acid extract are intensely coloured; others, especially those from lemon peels or from peels and pulp containing small amounts of biphenyl, require more careful purification, even if the chloroform layer and the sulphuric acid extract are colourless. After four or five treatments with sulphuric acid (six or seven for the less satisfactory extracts), check whether or not the extract is sufficiently pure and simultaneously determine its approximate biphenyl concentration. Place 0·01-ml portions of the chloroform layer in three separate test-tubes, and add to each 0·09, 0·14 or 0·19 ml of acetic acid reagent. Add to the contents of each tube 5 ml of sulphuric acid reagent, as described for the preparation of the standard graph. Measure the optical densities.

(*a*) If the colours produced are not clear blue, purify the extract an additional two or three times, and repeat the preliminary determination.

(*b*) If the colours of the solutions in the three test-tubes are blue and their optical densities are about the same, the extract is sufficiently pure and its biphenyl concentration is appropriate for the determination.

(*c*) If the colours produced are intense—blue or turning from violet to blue—and the optical densities increase for the larger amounts of acetic acid reagent, then the extract is sufficiently pure, but needs to be diluted. Take less than 0·01 ml of extract, *e.g.*, 0·005 or 0·0025 ml, until the optical densities obtained with increasing amounts of acetic acid reagent are about the same. Note the required degree of dilution.

(*d*) If the colours are not discernible visually or are only faintly blue or violet, the extract needs to be concentrated and the remaining impurities interfering with the colour reaction eliminated. (Extracts purified four or five times need to be purified a further two or three times before concentration.) Place 1 ml of the chloroform layer in a 10-ml graduated tube, add 1 ml of acetic acid reagent, and concentrate to 0·1 ml as described under "Procedure for determining Biphenyl." Add a further 1 ml of acetic acid reagent, and concentrate once more to 0·1 ml. Adjust the volume with acetic acid reagent to 0·5 ml for pulp extracts or 2 ml for peel extracts. Place in two separate test-tubes 0·10-ml portions of the mixture (0·30 ml if the colour is extremely faint), add to the contents of one tube 0·10 ml of acetic acid reagent, and then introduce into each tube 5 ml of sulphuric acid reagent as described for the preparation of the standard graph. If the colour is not clear, proceed as indicated under (*a*) above. Measure the optical densities (see (*b*) and (*c*)), and then calculate the volume of extract to be concentrated in order to make 5 or 10 ml of mixture.

PROCEDURE FOR DETERMINING BIPHENYL—

According to the approximate concentration of biphenyl in the extract determined under (*b*), (*c*) or (*d*) above, proceed appropriately as described below.

(*b*) Place 1 ml of extract in a 10-ml graduated tube, add 9 ml of acetic acid reagent, insert the stopper in the tube, and mix. Measure the optical densities corresponding to 0·05, 0·10 and 0·15 ml of the mixture (0·10, 0·20 and 0·30 ml if the colours are extremely faint) as described for the preparation of the standard graph. The colours obtained must be clear, and the optical densities should rise proportionally with the amounts of the mixture taken for the determination. Calculate the amount of biphenyl in 0·10 ml of the mixture.

(*c*) Place in a 10-ml graduated tube the required volume of extract, *e.g.*, 0·50 or 0·25 ml, adjust the volume to 1 ml with chloroform, add 9 ml of acetic acid reagent, insert the stopper in the tube, and mix; continue as under (*b*).

(*d*) Introduce by pipette into a 10-ml graduated tube a maximum of 5 ml of extract. Add 1 ml of acetic acid reagent, and insert the concentration adaptor (see Fig. 2) in the tube. Place the tube in a beaker containing a depth of 2 to 3 cm of water maintained at 50° to 60° C. Connect the adaptor to a vacuum pump, and carefully reduce the volume to about 1·0 ml. Close the connection to the vacuum pump, and add through the inlet a maximum of 5 ml of extract. Rinse the inlet with a few drops of chloroform, insert the stopper, and again reduce the volume to 1·0 ml. Continue this process until the required volume of extract has been introduced. Concentrate finally to 0·1 ml. Add 1 ml of acetic acid reagent, and concentrate once again to 0·1 ml. Remove the adaptor from the tube, and use 1 or 2 ml of acetic acid reagent to rinse the inside of the adaptor and the capillary; collect the rinsings in the graduated tube. Add 0·5 or 1·0 ml of chloroform, and adjust the volume with acetic acid reagent to 5 or 10 ml, respectively (see (*d*) under "Preliminary Determination"). Insert the stopper in the tube, and mix; continue as under (*b*).

CALCULATION—

Calculate the biphenyl content in the peel and in the pulp from the relation—

$$\text{Biphenyl content} = \frac{a \times 10 \times b \times 50}{c \times d} \text{ p.p.m.}$$

where a = micrograms of biphenyl in 0·10 ml of mixture,
b = volume of prepared mixture in millilitres,
c = volume of extract in millilitres taken for the preparation of the mixture and
d = weight of peel or pulp in grams taken for determination.

Calculate the biphenyl content of the whole fruit.

DISCUSSION OF THE METHOD

SENSITIVITY—

The method permits the determination of 0·06, 1·0 and about 0·3 p.p.m. of biphenyl in the pulp, peel and the whole fruit, respectively. The sensitivity can be increased by preparing from the concentrated extract a smaller amount of mixture or by using a larger sample of peel or pulp.

ACCURACY—

Biphenyl was determined in essential oils, peel and pulp of untreated fruit to which known amounts of biphenyl had been added (see Table I); in several samples originating from the same mixture of peels of fruit treated with biphenyl (see Table II), in several average samples of peels successively removed from the same batch of ten biphenyl-treated fruits (see Table III).

The results show that deviations are slight and remain within the limits of experimental error.

TABLE I

RECOVERY OF BIPHENYL

Sample	Biphenyl added, mg	Biphenyl found, mg	Difference, mg	Error, %
Orange essential oil	0·00	0·00	0·00	0·0
Orange essential oil	4·00	3·80	−0·20	−5·0
Orange essential oil	17·90	17·20	−0·70	−4·0
Orange essential oil	20·40	20·00	−0·40	−1·9
Orange essential oil	55·70	55·00	−0·70	−1·2
Orange peel	0·00	0·00	0·00	0·0
Orange peel	8·00	8·15	+0·15	+1·9
Orange peel	50·00	50·00	0·00	0·0
Lemon peel	0·00	0·00	0·00	0·0
Lemon peel	5·00	4·85	−0·15	−3·0
Lemon peel	8·00	7·80	−0·20	−2·5
Grapefruit peel	0·00	0·00	0·00	0·0
Grapefruit peel	8·00	7·90	−0·10	−1·2
Grapefruit peel	20·00	20·00	0·00	0·0
Orange pulp	0·00	0·00	0·00	0·0
Orange pulp	0·80	0·80	0·00	0·0
Lemon pulp	0·00	0·00	0·00	0·0
Lemon pulp	0·50	0·48	−0·02	−4·0
Grapefruit pulp	0·00	0·00	0·00	0·0
Grapefruit pulp	0·40	0·41	+0·01	+2·5

VALIDITY—

Distillation—Most of the biphenyl collected during distillation of a mixture of biphenyl and water was found in the first 25 ml of distillate (see Table IV). The amount of water, from 300 to 2000 ml, does not affect the results.

Moreover, it was observed that the amounts of biphenyl liable to be found in fruit samples are completely collected in the first 100 ml of distillate (see Table V). The times for complete distillation of peel and pulp samples are about 20 and 40 minutes, respectively.

TABLE II

DETERMINATION OF BIPHENYL IN TREATED ORANGE PEELS

Four samples taken from the same mixture of peels

Sample No.	1	2	3	4	Average
Biphenyl found, mg	40·50	41·13	39·50	40·50	40·41
Deviation from average value, mg ..	+0·09	+0·72	−0·91	+0·09	
Deviation from average value, % ..	+0·22	+1·77	−2·22	+0·22	

TABLE III

DETERMINATION OF BIPHENYL IN TREATED CITRUS PEELS

Three average samples taken from the same batches of 10 fruits

	Biphenyl found in—		
Sample No.	orange peels, mg	lemon peels, mg	grapefruit peels, mg
1	17·48	24·8	11·2
2	17·48	26·3	11·2
3	17·48	26·7	10·8

TABLE IV

RECOVERY OF BIPHENYL IN SUCCESSIVE 25-ml FRACTIONS OF DISTILLATE

Distillation of a mixture of water and biphenyl

	Biphenyl found in fraction No. —						
Biphenyl added, mg	I, mg	II, mg	III, mg	IV, mg	V, mg	VI, mg	total, mg
20·0	18·80	0·94	0·12	0·00	0·00	0·00	19·86
50·0	48·75	1·16	0·28	0·00	0·00	0·00	50·19
100·0	78·75	16·25	5·00	1·00	0·08	0·00	101·08

Purification with 95·2 *per cent. w/w sulphuric acid*—Conflicting opinions exist among workers[1,4,5,7,8,10] about the eventual loss of biphenyl owing to the action of the concentrated sulphuric acid used to remove interfering substances. According to Koether,[10] biphenyl is lost owing to sulphonation, and the loss increases with time of shaking. Rajzman[9] noted that biphenyl, detectable by the colour reaction, disappeared rapidly when a large amount of sulphuric acid was added to the biphenyl dissolved in a small amount of acetic acid or ethanol; this was attributed to sulphonation. On the other hand, during this investigation no loss of biphenyl was observed after rigorous purification of three volumes of chloroform extract by one volume of concentrated sulphuric acid.

That no biphenyl was lost seemed surprising; assuming that this was due to particular conditions empirically established for the clean-up procedure, the effect of 98 per cent. w/w sulphuric acid acting under various conditions on biphenyl dissolved in chloroform was studied. It was found that some biphenyl was lost, and a number of conclusions were drawn.

(1) Loss of biphenyl depends on the ratio between the volumes of concentrated sulphuric acid and chloroform; it increases with an increase in the ratio (see Table VI).

(2) Loss of biphenyl increases with shaking time and is accelerated by repeatedly replacing the concentrated sulphuric acid by fresh acid (see Table VII). The influence of shaking time on the loss of biphenyl was not constant.

(3) Loss of biphenyl depends on the concentration of the sulphuric acid, and decreases with increased dilution (see Table VIII).

TABLE V

RECOVERY OF BIPHENYL IN SUCCESSIVE 100-ml FRACTIONS OF DISTILLATE

	Biphenyl added, mg	Biphenyl found in fraction No.—			
		I, mg	II, mg	III, mg	total, mg
Untreated fruit—					
Orange pulp	0·40	0·40	0·0	0·0	0·40
Lemon pulp	0·40	0·38	0·0	0·0	0·38
Grapefruit pulp	0·80	0·78	0·0	0·0	0·78
Orange peel	10·00	9·85	0·0	0·0	9·85
Orange peel	20·00	20·00	0·06	0·0	20·06
Lemon peel	1·60	1·66	0·0	0·0	1·66
Lemon peel	4·00	4·00	0·0	0·0	4·00
Grapefruit peel	1·60	1·60	0·0	0·0	1·60
Grapefruit peel	20·00	19·90	0·04	0·0	19·94
Treated fruit—					
Orange pulp	—	0·45	0·0	0·0	0·45
Lemon pulp	—	0·28	0·0	0·0	0·28
Grapefruit pulp	—	0·12	0·0	0·0	0·12
Orange peel	—	32·60	0·09	0·0	32·69
Orange peel	—	35·44	0·0	0·0	35·44
Lemon peel	—	3·36	?	0·0	3·36
Lemon peel	—	10·15	0·0	0·0	10·15
Grapefruit peel	—	5·04	0·0	0·0	5·04
Grapefruit peel	—	6·18	0·02	0·0	6·20

TABLE VI

EFFECT OF THE RATIO BY VOLUME OF SULPHURIC ACID TO CHLOROFORM ON LOSS OF BIPHENYL

Biphenyl (300 mg per ml) in chloroform shaken for 15 minutes with 98 per cent. w/w sulphuric acid

Ratio	50 to 1	20 to 1	10 to 1	5 to 1	2 to 1	1 to 1	1 to 3
Loss of biphenyl, %	100·0	100·0	95·0	90·0	85·0	5·0	0·0

TABLE VII

EFFECT OF SHAKING TIME ON LOSS OF BIPHENYL

Biphenyl (10 mg) in 30 ml of chloroform shaken with 10 ml of 98 per cent. w/w sulphuric acid replaced every 10 minutes

Shaking time, hours	4	8	12	16
Loss of biphenyl, %	0	20	40	50

TABLE VIII

EFFECT OF WATER CONTENT OF SULPHURIC ACID ON LOSS OF BIPHENYL

Biphenyl (10 mg) in 30 ml of chloroform shaken with 10 ml of sulphuric acid replaced every 10 minutes; shaking time 12 hours

Concentration of sulphuric acid, % w/w	98·0	97·5	97·0	96·4	95·8	95·2
Loss of biphenyl, %	40	25	17	13	0	0

The 95·2 per cent. acid chosen lends itself well to the clean-up procedure. Less concentrated acids can be used, but the more dilute the acid the more highly coloured becomes the chloroform layer.

It is possible that the differences of opinion mentioned above are due, in part at least, to the fact that the sulphuric acids used by various workers, although concentrated, were not exactly similar.

Elimination of the remaining interfering substances—In some extracts, particularly from lemon peels, interfering substances reacting in part with formaldehyde were found to persist despite prolonged treatment with sulphuric acid, washing with sodium hydroxide solution and distilled water, and treatment with potassium permanganate.

This difficulty of eliminating interfering substances has also been reported by Gunther and Blinn[11] in the spectrophotometric determination of biphenyl[8]; despite a rigorous clean-up treatment, they found that a slight and variable interference resulted from the presence of *p*-cymene from the citrus oil.[11]

It is possible that the impurities interfering with the colour reaction are also formed, in part at least, by *p*-cymene, of which they possess certain properties.

It has been noted that these impurities dissolved in a small amount of acetic acid reagent (or acetic acid) are eliminated by evaporating the solvent. No loss of biphenyl was found to take place during the evaporation of acetic acid reagent as long as a small amount remained.

Presence of o-*phenylphenol*—It should be noted that *o*-phenylphenol, which is used as a fungicide, may be found in the extract. With sulphuric acid, traces of formaldehyde and ferric iron, *o*-phenylphenol gives a pink colour.[9] Nevertheless, its presence in the extract does not interfere with the determination of biphenyl, since it disappears quickly during the treatment with sulphuric acid.

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An Improved Chromatographic Method for determining Trace Elements in Foodstuffs

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The quantitative paper-chromatographic determination of copper, cobalt, nickel, molybdenum and manganese in foodstuffs is described. The extraction of the elements after ashing the material has been investigated. The elements are separated as their chlorides on slotted chromatography paper, and are identified colorimetrically. The concentrations are determined by reflectance measurements on the papers, and the results are calculated with the aid of prepared standard graphs. As little as 0·05 μg of the elements can be determined.

THE application of paper chromatography to separating and determining trace elements in foodstuffs has recently been reviewed and extended by some investigators.[1,2,3,4] The elements are separated and then determined colorimetrically[1,2] or by visual comparison with known standards on the papers after spraying with suitable reagents.[2] The determination has also been carried out by automatic densitometry. This investigation was undertaken in an endeavour to apply chromatography on Whatman No. 1 (type CRL/1) paper, as used by Hunt, North and Wells[1] and Duffield,[2] to the routine determination of trace elements in foodstuffs. During this work several modifications were introduced, and the determinations were carried out by a simplified technique with use of an E.E.L. mark III Reflectometer Head (Evans Electroselenium Ltd.). The elements determined are copper, cobalt, nickel, manganese and molybdenum.

EXPERIMENTAL

The ashing and leaching of various types of analytical samples came under close investigation. The different ash contents cause some difficulty, since a large volume of solvent is required for extracting the elements from material having a high ash content before they can be applied on the paper. Cobalt, nickel and molybdenum, normally present at low concentrations, would not be detected in these circumstances. Coulson, Davies and Luna[4] used a wet-ashing technique; they then filtered and extracted with dithizone to concentrate the elements. This proved time-consuming, and ashing with concentrated nitric acid was then investigated.[5] This gives an extremely good ash, but a tendency to spatter makes it of doubtful value in work on trace elements; it also necessitates the conversion of the nitrates formed to the chlorides. Webb, Stevens and Hallas[3] used an extraction technique based on the fact that the chlorides of the elements are soluble in 1 per cent. hydrochloric acid in acetone; it could not, however, be incorporated in the proposed technique, owing to the small volumes required for spotting on the papers. The main interfering elements encountered in large amounts are calcium, magnesium, iron, potassium and silicates. In the proposed method, centrifugation is used to remove silicates and other ashing debris, and all evaporations are carried out in the original ashing dishes. This procedure requires the minimum of equipment, and, after ashing overnight, the medium can be prepared for chromatography in only 6 hours. The final precipitate is dissolved in a minimum of 2 ml of diluted hydrochloric acid (1 + 1).

The two solvent systems used are—

1. For separating copper, cobalt, nickel and manganese, ethyl methyl ketone, concentrated hydrochloric acid and water (15:3:2); approximately 30 minutes are required for development. When much copper and iron are present tailing of the copper takes place, but can be prevented by changing the solvent ratio to 15:3·5:1·5.
2. For separating molybdenum and zinc, n-butanol and 4 N hydrochloric acid (15 + 6); 2 hours are required for development.

The determination of copper, cobalt and nickel with rubeanic acid follows that laid down by Hunt, North and Wells[1] and used by Duffield.[2] This allows the use of a graded distribution in the standards from 0·05 to 1·0 μg, in steps of 0·1 μg. Amounts of cobalt less than 0·05 μg are best determined with 2-nitroso-1-naphthol, which, according to Bellucci,[6] is more sensitive than the 1-nitroso-2-naphthol used by Coulson, Davies and Luna.[4] The greater sensitivity of 2-nitroso-1-naphthol was not confirmed by Yoe and Barton,[7] but in this investigation it gave a more intense colour on the paper. Manganese is determined in the same solvent and developed with formaldoxime, as described by Webb, Stevens and Hallas.[3] Time is a critical factor in these analyses, particularly in the determination of manganese, the colour of which tends to fade rather quickly in air. The times laid down in the proposed method are essential for reproducibility and for comparison with standards.

The separation of molybdenum and zinc is best carried out in solvent mixture 2. The zinc travels as a band, which shows up well with the Zincon reagent or 1-nitroso-2-naphthol-4-sulphonic acid. It is not possible to determine zinc quantitatively on paper, since the band produced is so concentrated that even with millimicrogram amounts it leads to erroneous comparisons with standards. It is best determined colorimetrically after the spots have been eluted from the paper. Molybdenum is determined with dithiol as indicator, which is normally used in conjunction with thioglycollic acid in 1 per cent. w/v sodium hydroxide solution.[8] This was found to be impracticable, as the high concentration of iron present forms a purple thioglycollate complex that tends to mask and interfere with the normal development of the molybdenum - dithiol complex. Bingley[8] reported that the presence of iron increased the sensitivity of the molybdenum - dithiol complex, but he again incorporated the thioglycollic acid. He also reported that a concentration of 4 N hydrochloric acid was necessary. In the proposed method the dithiol is dissolved in ether and sprayed directly on the dry paper after chromatography. After the paper has again been dried and then sprayed with N hydrochloric acid, iron shows as a greyish spot that does not interfere with the colour-density determination of molybdenum; hydrochloric acid helps to fix and intensify the green dithiol complex.

When visual analysis is applied to a sample, standards must be run at the same time. This is time-consuming and, when more than 50 p.p.m. of maganese are present, it can lead to large errors because of the dilution factors involved. These errors can be avoided by employing reflectance measurements with an E.E.L. mark III Reflectometer Head and Unigalvo type 20. The head is used without the diffusing glass or lens, and, by selecting appropriate filters for the colours developed on the paper, the percentage reflectance can be measured. By this means standard charts are prepared of concentration (0·05 to 0·5 μg) against percentage reflectance. For the reflectance measurements masks are used that limit the reading to the area of the spot. Brass plates with slits (*a*) 13 mm × 20 mm and (*b*) 13 mm × 15 mm were made to fit the head, 13 mm being the width of each strip of the CRL chromatography paper. Mask (*a*) is used for molybdenum, manganese and nickel, and mask (*b*) for copper and cobalt.

METHOD

APPARATUS—

Vitreosil basins.

Centrifuge tubes, 15 *ml.*

Stoppered glass tubes.

REAGENT—

Hydrochloric acid, diluted (1 + 1).

PREPARATION OF SAMPLE—

Ash 20 g of the sample overnight at 550° C in a vitreosil basin. Allow to cool, and add 5 ml of diluted hydrochloric acid (1 + 1). Evaporate on a steam-bath, and heat on a hot-plate for 30 minutes. Repeat the hydrochloric acid addition and evaporation, and then add 5 ml of hot diluted hydrochloric acid (1 + 1), heat, and transfer to a 15-ml centrifuge tube, washing in all debris with not more than 5 ml of diluted hydrochloric acid (1 + 1). Place the tube in a boiling-water bath for 30 minutes, spin in a centrifuge, and then return the supernatant liquid to the vitreosil basin. To the precipitate in the tube add 5 ml of diluted hydrochloric acid (1 + 1), and again place in a boiling-water bath for 30 minutes;

then spin in a centrifuge, and add the washings to the original supernatant liquid. Repeat the washing. Evaporate the combined solutions to dryness on a steam-bath, and dissolve the residue in 2 ml of diluted hydrochloric acid (1 + 1); if necessary, heat gently to dissolve. Transfer the solution to a stoppered glass tube. Prepare (1 + 4), (1 + 9) and (1 + 19) dilutions of portions from the stoppered glass tube, and use 0·01 ml of these solutions for spotting on the paper.

PAPER CHROMATOGRAPHY

APPARATUS—

Petri dishes, 5 inch—For covering the beakers.

Capillaries—Calibrated to deliver 0·01 or 0·02 ml.

Whatman No. 1 chromatography paper (type CRL/1)—This paper allows the application of 10 samples at a time.

A swivel mounted cradle on which the paper can be mounted for spraying purposes.

E.E.L. mark III Reflectometer Head.

E.E.L. Unigalvo type 20.

PROCEDURE—

Apply 10-μl spots of sample solution on the paper, and dry in a current of warm air to give a spot 1 cm in diameter on the baseline, which is approximately 1·5 cm from the bottom of the paper. Join the ends of the paper to form a cylinder, and place in a 500-ml beaker containing 20 ml of the appropriate solvent; cover with a Petri dish, and develop by an ascending-solvent technique.

DETERMINATION OF COPPER, COBALT AND NICKEL

REAGENTS—

Solvent mixture—Ethyl methyl ketone, concentrated hydrochloric acid and water in the ratio 15:3:2.

Rubeanic acid solution—A 0·1 per cent. w/v solution in ethanol.

2-Nitroso-1-naphthol solution—Dissolve 0·05 g of the reagent in 10 ml of water and 0·5 ml of N sodium hydroxide with heating, and then dilute to 100 ml with water.

Ammonia solution, sp.gr. 0·88.

PROCEDURE—

Apply the sample as described above, and stand the paper in the beaker containing 20 ml of the solvent. Allow the solvent to diffuse to the top of the strips (30 minutes). Remove the paper, and dry in air for 5 minutes. Stand the dried paper in ammonia vapour for 10 minutes to neutralise, dry in a current of warm air, and then spray both sides with the rubeanic acid solution. The spots denoting copper, cobalt, and nickel can be read immediately; nickel (purple-blue, R_F 0·07), cobalt (orange-yellow, R_F 0·27) and copper (olive-green, R_F 0·48).

DETERMINATION OF MANGANESE

REAGENTS—

Solvent mixture—Ethyl methyl ketone, concentrated hydrochloric acid and water in the ratio 15:3:2.

Formaldoxime—Dissolve 20 g of paraformaldehyde and 55 g of hydroxylamine sulphate in boiling water, and dilute to 100 ml. Dilute 1 ml of this solution to 10 ml with water.

Ammonia solution, sp.gr. 0·88.

Sodium hydroxide, N.

PROCEDURE—

Run the chromatogram as for copper, cobalt and nickel. Dry the paper in air for 5 minutes, and neutralise in an atmosphere of ammonia for 10 minutes; dry, and spray with formaldoxime. Replace the paper in ammonia vapour for 5 minutes, then spray with N sodium hydroxide to fix the complex, and dry in a current of warm air. When the paper is dry, replace it in ammonia vapour for 5 minutes, and then read immediately. The colour can be re-intensified in ammonia vapour should it fade during reading. Manganese is denoted by a reddish brown colour (R_F 0·15). The time sequence is extremely important for this element.

DETERMINATION OF MOLYBDENUM

REAGENTS—

Solvent mixture—n-Butanol - 4 N hydrochloric acid mixture (15 + 6).

Dithiol solution—A 1 per cent. w/v solution in ether, prepared once a week.

1-*Nitroso-2-naphthol-4-sulphonic acid solution, 0·5 per cent. w/v in ethanol.*

PROCEDURE—

Apply the samples as stated previously, and develop in the butanol - hydrochloric acid solvent (2 hours). Remove the paper, and, for determining molybdenum, dry in a current of warm air. Spray with dithiol solution, dry, and spray with N hydrochloric acid. Molybdenum is denoted by an apple-green colour (R_F 0·55).

The presence of zinc may be detected by running a paper as for molybdenum, neutralising in ammonia vapour for 5 minutes, and spraying with 1-nitroso-2-naphthol-4-sulphonic acid solution. At levels as low as 0·1 μg on the paper, zinc appears as an orange band (R_F 0·88).

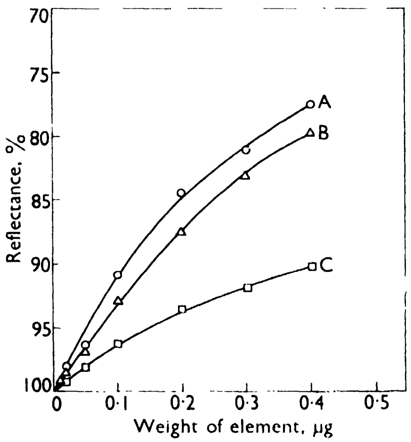

Fig. 1. Calibration curves: curve A, cobalt; curve B, nickel; curve C, copper

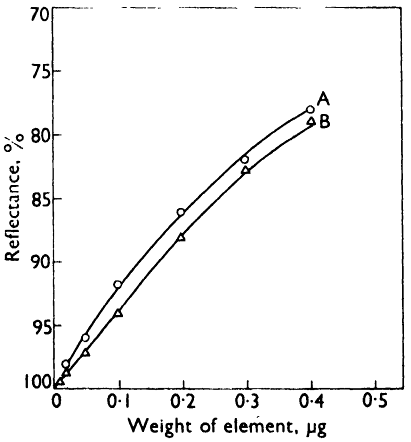

Fig. 2. Calibration curves: curve A, molybdenum; curve B, manganese

PREPARATION OF STANDARDS

Prepare a mixed synthetic standard containing copper, cobalt, nickel, molybdenum, manganese and zinc. Dilute to the required concentrations, evaporate the solutions, and carry out the extraction procedure described for the ashed material. Finally, dissolve in 2-ml portions of dilute hydrochloric acid (1 + 1) so that 10-µl portions contain 0·05, 0·1, 0·2, 0·3, 0·4 or 0·5 µg of the elements.

Run chromatograms of the standards, and read the reflectance with the red filter for copper, nickel and molybdenum and the blue filter for cobalt and manganese. Prepare standard curves of percentage reflectance against concentration (see Figs. 1 and 2).

TABLE I

ANALYSIS OF CEREALS

Cereal	Copper found		Cobalt found		Nickel found		Manganese* found		Molybdenum found	
	A, p.p.m.	B, p.p.m.	A, p.p.m.	B, p.p.m.	A, p.p.m.	B, p.p.m.	A, p.p.m.	B, p.p.p.	A, p.p.m.	B, p.p.m.
Oats ..	3·25	3·1	<0·05	0·02	1·5	2·9	62	54	0·33	0·38
Barley ..	5·0	6·5	<0·05	0·01	0·25	0·28	27	30	0·15	0·16
Oats ..	6·25	6·2	<0·05	0·05	6·75	7·2	90	98	0·35	0·44
Barley ..	6·75	7·2	<0·05	0·01	0·2	0·11	17	19	0·2	0·18
Oats ..	3·8	4·2	<0·05	0·01	0·15	0·17	40	33	0·25	0·44
Barley ..	4·2	4·1	<0·05	0·01	0·3	0·30	17	17	0·35	0·68
Oats ..	2·25	2·3	<0·05	0·03	0·7	1·10	44	32	0·28	0·33
Barley ..	3·25	2·8	<0·05	0·02	0·25	0·15	15	14	0·4	0·47

A = Chromatographic analysis. B = Spectrographic analysis.

* Manganese, which is present in large amount, requires a dilution of (1 + 19) to bring the final spot within the range of the reflectometer. Cobalt, molybdenum and nickel, present in only small amounts, are placed on the paper in 20-µl spots; this brings the reading on the reflectometer to a more acceptable range.

TABLE II

RECOVERY OF ELEMENTS FROM A SAMPLE OF OATS

Mean of three results

	Copper		Cobalt		Nickel		Manganese		Molybdenum	
Amount present in original material, µg	5·2	5·2	—	—	0·1	0·1	21	21	0·3	0·3
Amount added, µg ..	4·0	8·0	<0·01	<0·02	0·17	0·34	13	26	0·44	0·88
Total amount found, µg	8·6	15	<0·05	<0·05	0·4	0·6	35	51	0·6	0·9

TABLE III

CHROMATOGRAPHIC ANALYSIS OF FOODSTUFFS

Sample	Ash content, %	Copper found, p.p.m.	Cobalt found, p.p.m.	Nickel found, p.p.m.	Molybdenum found, p.p.m.	Manganese found, p.p.m.
Heavy hog fattening ration	7	22	<0·15	<0·15	<0·15	108
Sow and weaner mix ..	7·3	75	<0·1	<0·1	<0·1	78
	7·2	67	<0·15	<0·15	<0·15	81
Fishmeal	12·6	2	<0·15	<0·15	<0·15	11
	12·8	3	<0·15	<0·15	<0·15	11
Meat and bone meal ..	42	—	<0·5	<0·5	<0·5	17

DISCUSSION OF METHOD

Table I shows the results obtained on cereals by the proposed method. These results compare favourably with those by arc spectrography carried out by a separate investigator at the Johnstown Castle Soils Laboratory, Co. Wexford. The ashing and extraction techniques used in the latter method were those of Mitchell[9] and Farmer.[10] The value of the method is obvious when it is considered that 10 samples can be analysed by one operator for these five elements in 2 days, a minimum of space, equipment and chemicals being necessary. When determining manganese it is advisable to include a known standard on the paper for reading, together with the unknowns, as a check that the development procedure has been carried out properly. A high iron content in the original sample will tend to inhibit the separation of molybdenum, but, if the paper is left for at least 15 minutes in the solvent after completion of the run, a definite separation is obtained.

Table II shows recoveries carried out on a sample of oats expressed as the mean of three determinations. In the analysis of foodstuffs with a high ash content (see Table III) a large volume of diluted hydrochloric acid (1 + 1) is required for dissolving the extracted elements before they are placed on the papers. For meat and bone meal, which has a 42 per cent. ash, 10 ml of acid are required to dissolve the elements from 10 g of material. This raised the background limit of detection from 0·05 to 0·5 p.p.m.

The reflectance measurement operates within a range of 0·01 to 0·5 μg with a short deflection on the scale. Above 0·5 μg the curve levels off owing to supersaturation of the spot with the element. This can be a source of error when reading from the chart, as a change in reflectance of 1 division on the scale can amount to 0·02 μg for nickel and 0·03 μg for copper. This is not a serious error with an undiluted solution, but for manganese, which requires a dilution of (1 + 19) when present at the 100 p.p.m. level, the error can be ±6 per cent.

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SHORT PAPERS

"Water Minus" (H_2O^-) in Natural Glasses

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In fresh crystalline igneous rocks there is usually a clear-cut distinction between absorbed or loosely held water ($H_2O^{110° C}$) and that constitutionally held ($H_2O^{+110° C}$). The former rarely amounts to more than a few tenths of a per cent. by weight of the total assay.

Analyses of natural glasses reported in the literature show wide variations in the amount of "water minus." A figure of 3 per cent. is exceeded occasionally and is to be regarded as falling within the range of normality. The usual limit for total water is around 9 per cent. by weight. It appears that a sharp discrimination between absorbed and constitutional water cannot be made for such materials.

Carmichael[1] found that it was "apparently not possible to dry the . . . glass powders to constant weight at 110° C, there being a small and continuous loss in weight." Butler[2] also found that, although most of the "water minus" was evolved during the first 10 minutes of heating, loss of weight continued, even after 19 hours at the same temperature. In this laboratory samples of natural acid glasses have been observed to lose weight at temperatures substantially below 100° C, and it was decided to investigate the phenomenon of dehydration at low temperatures in more detail.

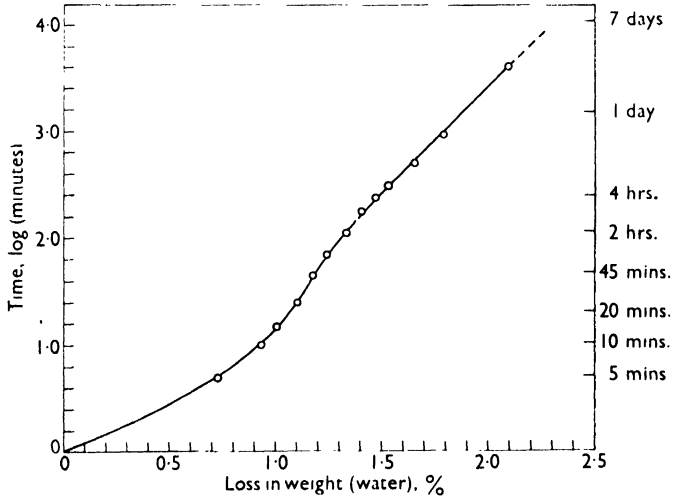

Fig. 1. Percentage by weight loss of water plotted against the logarithm of the duration of heating at 110° C for the glass separated from the Raasay pitchstone

A sample of the pitchstone from Raasay, Inner Hebrides, was crushed, and the glass, essentially free from crystalline matter, was separated from it. The pure glass was found to contain 7·15 per cent. by weight of total water. A portion was heated at 110° C on a Stanton recording thermobalance, and in Fig. 1 is shown a plot of the weight loss against the logarithm of the duration of heating. The experiment was continued for almost 70 hours, and it can be seen that, of the recorded water loss, only about half took place during the first hour. Although water was lost at a rate that continually diminished, the form the curve strongly suggests that an extension of the heating time would have resulted in the expulsion of more water.

A second experiment was carried out in which the Raasay glass was used, together with a sample of perlitic natural glass from Chaffee County, Arizona; for comparison, samples of a serpentine and of apophyllite were used. Each substance was dehydrated at temperatures progressively raised above the ambient in steps of 15° to 20° C, the sample being maintained at each temperature until the rate of water loss became extremely low. This usually took 12 to 24 hours. The results were plotted, and the graph is shown in Fig. 2. The Raasay glass lost 23 per cent. of its total water (1·65 per cent. by weight) at a temperature not exceeding 110° C, and the curve is smooth with only minor inflexions up to 650° C. The Chaffee perlite lost little

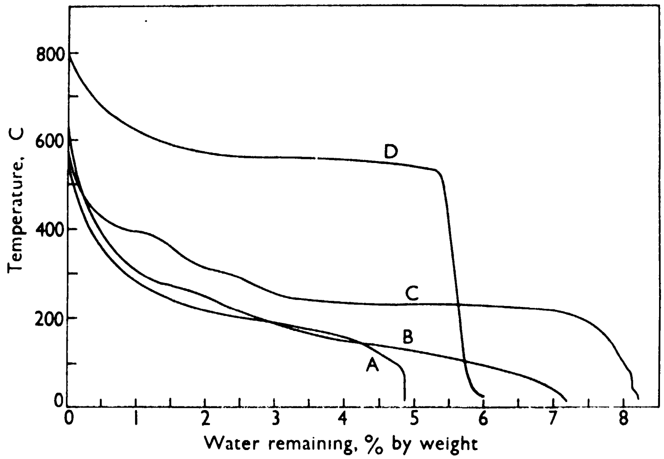

Fig. 2. Quasi-static dehydration curves showing differences in behaviour between hydrated glasses and hydrous minerals: curve A, Chaffee perlite (4·85 per cent. of water); curve B, Raasay glass (7·15 per cent. of water); curve C, apophyllite (16·35 per cent. of water); curve D, serpentine (12·02 per cent. of water). The last two minerals are plotted half scale

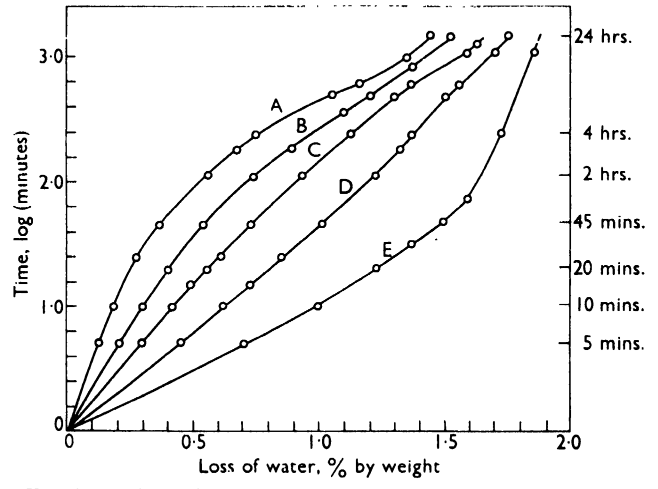

Fig. 3. Effect of grain size of the sample on the proportion of water evolved from Mull glass No. 78 at 110° C over 24 hours: curve A, 25 to 50 mesh; curve B, 50 to 100 mesh; curve C, 100 to 170 mesh; curve D, 170 to 300 mesh; curve E, <300 mesh

water at and below 110° C, and the curve is smooth and continuous through this region. Above 150° C the behaviour of this glass closely parallels that of the Raasay sample. Note that small variations in the temperature of dehydration would give differing results for the H_2O^- value. A variation in temperature of 10° C for instance could cause a difference in the H_2O^- value of ±0·15 per cent., by weight, of water.

Of the crystalline materials, the serpentine lost a little water below 110° C that can reasonably be described as absorbed or hygroscopic. Above 110° C there was comparatively little further loss before substantial dehydration set in at about 530° C. This typifies the relations found in many hydrous silicate minerals. The apophyllite lost only traces of water up to about 80° C, but above this temperature water began to be evolved that cannot be sharply separated from the main loss of water in the 200° to 400° C range.

In a third experiment, the effect of grain size of the sample on the final "water minus" figure was evaluated. A sample, No. (Lab.) 78, of a pitchstone from Mull, with a total water content of 7·28 per cent., was sifted into five fractions, 25 to 50 mesh, 50 to 100 mesh, 100 to 170 mesh, 170 to 300 mesh and that passing 300 mesh. Each fraction was dehydrated at 110° C for 24 hours on the thermobalance, the loss of weight with time being continuously recorded. The results were plotted and the graph is shown in Fig. 3; the co-ordinates were the same as those of the graph shown in Fig. 1. It can be seen that grain size has a profound effect on the "water minus" value whatever the duration of heating. Ironically, the differences between the various grain sizes are probably greatest at the classically recommended time of 1½ to 2 hours for determining "water minus." The differences between the curves are reduced with increased duration of heating, but even after 24 hours an error of up to 0·5 per cent. by weight of water can be expected.

In a fourth experiment, the equilibrium vapour pressures of several samples of hydrated glass were measured and were found to be appreciable at temperatures below 110° C. Sample No. 78 (7·28 per cent. of total water) separated from a pitchstone intrusion in the Loch Scridian district of Mull gave a water vapour pressure of 1·5 mm of mercury at 33° C, 24·6 mm at 58° C and 409 mm at 106° C. Another glass, No. 207 (4·0 per cent. of total water) from a similar intrusion, gave a vapour pressure of 0·1 mm at 31° C, 14·0 mm at 60° C and 308 mm at 110° C.

To test the possible effect of oxidation of ferrous iron in finely crushed glasses at 110° C over long periods, a sample of a natural glass containing 9 per cent. of FeO and 0·10 per cent. of total water was crushed through a 150-mesh sieve and heated at this temperature for over 30 hours. The sample showed no measurable increase in weight due to oxidation after this period.

It is evident that the conventional determination of "water minus" in natural hydrated glasses is an arbitrary procedure giving results that cannot be evaluated in terms of the content of hygroscopic water. The H_2O^- figure is extremely sensitive to variations in temperature, in the duration of the heating and in the grain size of the sample. When the experimental conditions are rigidly controlled the differences reflect complex constitutional variations. We would recommend that the procedure be retained as a useful rough measure of the proportion of more loosely held water present in the glass. In order that future determinations should attain comparability, the conditions detailed below should be observed—

(*a*) The grain size of the glass sample should be between 150 and 300 mesh, or, for bulk samples of glassy rocks for which this is not possible, the sample should be crushed through a 150-mesh sieve. In any event, the mesh of the sieves used should be reported.

(*b*) The sample should be heated for 24 hours rather than 1½ to 2 hours.

(*c*) The temperature during the heating should be as closely controlled as possible at 110° C. Oven temperatures are notoriously variable unless special precautions are taken.

(*d*) If heavy liquids have been used for separating the glass from extraneous material, the sample should be dried at room temperature in air and in no circumstances should it be heated. Nor, because of the relatively high water vapour pressures of some glasses at low temperatures, should they be stored in a desiccator.

(*e*) Water determinations should be carried out as soon as possible after the preparation of the sample.

(*f*) The sample should be stored at room temperature in a sealed container of the minimum practical volume.

Numerous species and varieties of hydrated crystalline silicates are found in hydrothermally altered igneous rocks, metamorphic rocks and in sediments. Such rocks may be expected frequently to contain minerals that evolve water at low temperatures. The routine described above should therefore be followed carefully when materials of these kinds are being examined.

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The Determination of Carbon Dioxide by Non-aqueous Titrimetry

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THE method described by Blom and Edelhausen[1] for determining carbon dioxide, involving absorption in acetone or pyridine followed by titration with sodium methoxide in methanol - pyridine solution with thymol blue as indicator, has several disadvantages. These include the obnoxious nature of pyridine, the necessity for purifying it before use[2] and the inflammability of acetone, particularly since it may be used in the vicinity of combustion furnaces. Moreover, the high proportion of methanol in the titrant interferes with the sharpness of the end-point because of its acidic behaviour in non-aqueous media.

Fritz[3] has discussed the merits of other compounds, such as formdimethylamide, ethylenediamine and n-butylamine, as solvents for acidic substances in general, and concludes that formdimethylamide is an excellent solvent for many acids and for their sodium and potassium salts, is suitable for use in titrations with visual indicators and is superior to either ethylenediamine or n-butylamine.

Fritz and Keen[4] have proposed alkaline methoxide in benzene - methanol solution as titrant for acids dissolved in non-aqueous media. The amount of methanol is kept to the minimum required to give a homogeneous solution and, since potassium methoxide requires less methanol for this, it gives sharper end-points than sodium methoxide.

Formdimethylamide has been found to absorb carbon dioxide very readily. It is commercially available of sufficient purity to require no further treatment and is much less inflammable than acetone. The use of potassium methoxide in benzene - methanol as titrant and the replacement of thymol blue by the one-colour indicator, thymolphthalein, has considerably improved the end-point. It has also been possible to simplify the carbon dioxide absorption apparatus by making use of a magnetic stirrer.

The method described has been used for determining carbon dioxide produced by a variety of materials and is applicable to the continuous determination of carbon dioxide, provided that the carbon dioxide is always purified before absorption. Purification is necessary because water vapour (formed during the combustion of organic compounds), hydrogen sulphide (formed by the action of acid on minerals), etc., adversely affect the end-points and give results that are too high owing to their acidic behaviour in formdimethylamide.[5]

STANDARDISATION OF POTASSIUM METHOXIDE

Standard 0·1 N potassium methoxide in benzene - methanol is now commercially available, but as other concentrations may be required dependent on the determination to be carried out and since slight deterioration may occur on storage, it is advisable to standardise the solution before use. A convenient concentration for many determinations is 0·02 N.

PROCEDURE—

Place 10 ml of formdimethylamide in a 25-ml conical flask, add two drops of thymolphthalein (0·1 per cent. w/v in absolute methanol) and exactly neutralise the solvent (which contains a trace of carbon dioxide absorbed from the atmosphere) with the methoxide to the faint blue colour of the indicator. Add about 10 mg of pure benzoic acid, accurately weighed, and again, without delay, titrate to the full blue colour.

1 ml of 0·02 N potasium methoxide = 2·441 mg of benzoic acid.

Alternatively, the titrant can be standardised by titrating the carbon dioxide expelled from known weights of pure sodium hydrogen carbonate by heating and absorbed in formdimethylamide (after removal of moisture by passage through calcium chloride saturated with carbon dioxide) in the absorption apparatus described below.

The results obtained by each method are shown in Tables I and II; they indicate a satisfactory degree of precision and agreement.

TABLE I

STANDARDISATION OF 0·02 N POTASSIUM METHOXIDE AGAINST BENZOIC ACID

Benzoic acid, mg	Potassium methoxide titre, ml	Normality of potassium methoxide
9·216	3·830	0·01970
7·134	2·950	0·01980
8·213	3·395	0·01978
7·130	2·935	0·01990
6·914	2·865	0·01975

TABLE II

STANDARDISATION OF 0·02 N POTASSIUM METHOXIDE AGAINST CARBON DIOXIDE PRODUCED BY HEATING SODIUM HYDROGEN CARBONATE

Sodium hydrogen carbonate, mg	Calculated weight of carbon dioxide, mg	Potassium methoxide titre, ml	Normality of potassium methoxide
10·131	2·652	6·100	0·01975
8·474	2·218	5·070	0·01990
6·930	1·813	4·165	0·01981
8·988	2·353	5·400	0·01981

ABSORPTION AND TITRATION OF CARBON DIOXIDE

The absorption - titration apparatus is shown in Fig. 1 and consists of a wide-necked 25-ml conical flask having a three-hole stopper through which passes the carbon dioxide inlet, A, the 5-ml burette, B, and the exit tube, C. A two-way stopcock, D, on the inlet tube permits the combustion apparatus (not shown) to be flushed with nitrogen (or oxygen during combustions) without contaminating the solvent in the flask. A guard tube containing soda lime on the exit tube, C, prevents ingress of carbon dioxide from the atmosphere. The titrant in the burette is also protected from the atmosphere, and the tip of the burette is immersed in the absorption liquid, which is stirred continuously during absorption of carbon dioxide by a magnetic stirrer.

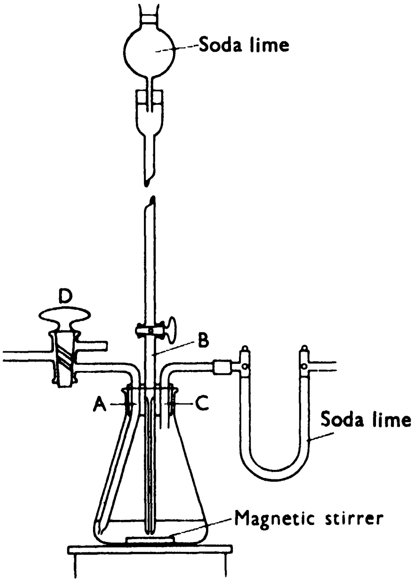

Fig. 1. Apparatus for absorption and titration of carbon dioxide

PROCEDURE—

Place 10 ml of formdimethylamide in the flask, and add two drops of thymolphthalein. Turn the stop-cock, D, so that the gas is ejected, and pass purified air, nitrogen or oxygen through the combustion system for 2 or 3 minutes to expel traces of carbon dioxide. Turn the stopcock to connect the combustion system with the flask, and pass the flushing gas through for a similar length of time. Neutralise the formdimethylamide with standard potassium methoxide to the faint blue colour of the indicator after isolating the flask from the combustion train by the stopcock. Turn the stopcock to reconnect the combustion train with the flask, and generate the carbon dioxide, keeping the absorbent stirred. The total rate of flow should not exceed approximately 60 ml per minute, otherwise absorption may be incomplete.

When all the carbon dioxide has been displaced, flush the combustion train into the absorbent, isolate the train by means of the stopcock, and titrate with standard potassium methoxide to the faint blue of the indicator.

Several determinations may be carried out without renewing the formdimethylamide, and, if desired, the carbon dioxide can be titrated during generation as the indicator gives a visual check on the absorption.

1 ml of 0·02 N potassium methoxide ≏ 0·44 mg of carbon dioxide

The method applied to a number of different materials gave the results shown in Tables III and IV.

TABLE III

DETERMINATION OF CARBON DIOXIDE IN MINERALS

Sample	Normality of potassium methoxide	Weight of sample, mg	Carbon dioxide— found, %	determined gravimetrically, %
Limestone	0·02	5·238	42·02	41·99
		6·141	42·10	
Dolomite	0·02	7·130	41·70	41·69
		5·002	41·80	
		8·130	41·82	

TABLE IV

DETERMINATION OF CARBON AS CARBON DIOXIDE IN ORGANIC COMPOUNDS AND A STEEL

Sample	Normality of potassium methoxide	Weight of sample, mg	Carbon found, %	Carbon present %
Benzoic acid (OAS)	0·1	13·263	68·68	68·84
		15·142	68·80	
Acetanilide (OAS)	0.1	12·163	71·00	71·08
Steel B.C.S. No. 8A	0·02	12·630	2·63	2·59
		15·144	2·57	

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The Chromatographic Separation of Chlorinated Propionic and Acetic Acids

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COLUMN chromatography has been investigated as a possible means of separating chlorinated propionic and acetic acids. The best results were obtained with cellulose powder as the column support, 0·1 N sulphuric acid as stationary phase and a 5 per cent. solution of analytical-reagent grade diethyl ether in light petroleum (boiling range 40° to 60° C) as the mobile phase. Lower proportions of diethyl ether improve the resolution and higher proportions elute the acids more quickly; 5 per cent. was selected as being the best practical compromise.

PROCEDURE—

Weigh out 30 g of standard grade ashless cellulose powder, and mix it thoroughly with 10 ml of 0·1 N sulphuric acid, the cellulose being added to the acid in small portions at a time. Plug the lower end of a chromatographic column, approximately 50 cm long and 1·6 cm internal diameter, with cotton-wool, and fill the column with a 5 per cent. solution of diethyl ether in light petroleum (boiling range 40° to 60° C). Add the prepared cellulose in small portions at a time, agitate thoroughly after each addition to remove the air bubbles, and pack down to a uniform tightness, so that, when all the cellulose has been added, the height of the packed column is about 35 cm.

Accurately weigh about 0·1 g of sample, place it on the column, and elute with the mobile phase; collect 10-ml fractions at the bottom of the column, preferably with an automatic fraction collector. To each fraction add 5 ml of a neutral 0·4 per cent. solution of phenolphthalein in ethanol, and titrate with 0·1 N potassium hydroxide from a microburette. Plot the titre of each fraction against the fraction number.

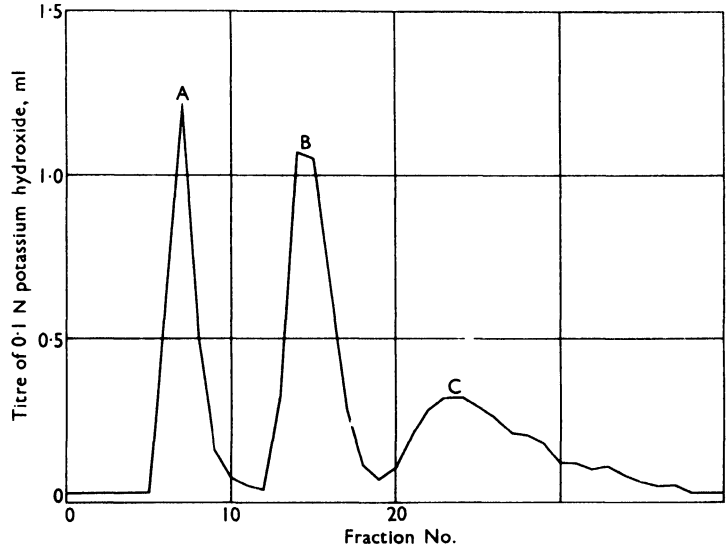

Fig. 1. Separation of chloropropionic acids: A, trichloro- *plus* dichloropropionic acids; B, monochloropropionic acid; C, propionic acid

RESULTS—

A typical experimental curve for the chloropropionic acids is shown in Fig. 1. 2,2-Dichloro- and 2,2,3-trichloropropionic acids are not separated from each other, but in the materials we used trichloropropionic acid was present only in extremely small amounts. For complete separation of monochloropropionic acid from unchlorinated propionic acid, not more than 20 per cent. of the unchlorinated acid should be present.

TABLE I

COMPARISON OF CHROMATOGRAPHIC METHOD WITH THE MERCURIC NITRATE METHOD

Sample No.	Monochloropropionic acid found by proposed method, %	Dichloropropionic acid, including any trichloropropionic acid, found by proposed method, %	Dichloropropionic acid found by mercuric nitrate method, %
1	83·5	14·2	11·4
2	83·3	14·2	11·8
3	55·3	36·4	37·1
4	55·1	40·7	41·1
5	42·2	51·1	51·3
6	15·4	74·7	76·4
7	13·3	80·3	82·0
8	8·9	80·8	80·3

If it is desired to use the separation as a means of determining the individual acids, the sum of the titres corresponding to the position of each peak is obtained, and the total titre for each is calculated as the appropriate acid. Table I shows some results by this method compared with some results obtained for dichloropropionic acid by the mercuric nitrate method.[1]

When the technique was applied to the chlorinated acetic acids, trichloroacetic acid and dichloroacetic acid were eluted together between fractions 15 and 30, and nothing else was eluted up to fraction 60. The chief use of the method was for determining dichloroacetic acid in monochloroacetic acid, trichloroacetic acid being almost completely absent. In this determination it was possible to use much larger weights of sample, and Table II shows the degree of reproducibility obtained.

TABLE II

DETERMINATION OF DICHLOROACETIC ACID IN CHLORINATED ACETIC ACID

Sample No.	Dichloroacetic acid found, %	Sample weight, g
1	8·4, 9·0	2·17, 1·38
2	10·1, 10·0	1·34, 1·90
3	5·2, 5·0	2·01, 1·99
4	2·0, 2·0	1·83, 2·21
Sodium salt	0·20, 0·11	1·06, 1·37

For crystalline samples, the sample is first dissolved in the minimum amount of water before it is placed on the column; for the sodium salts of the acids, it is necessary to dissolve in water and then add 1 ml of 10 N sulphuric acid.

We thank the Management of Lankro Chemicals Limited for permission to publish this paper and Dr. A. Gill and Mr. H. Higson of the Research Department for their co-operation.

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The Determination of Total Sulphur in Silicate Rocks by Wet Oxidation

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IN the usual method[1] for determining total sulphur in rocks, the sample is subjected to an oxidising fusion with a mixture of sodium carbonate and nitrate; the cooled melt is leached and filtered, the filtrate acidified and the sulphur, now present as sulphate, is precipitated as barium sulphate after removal of silica by acid fixation. This method is time-consuming and is associated with relatively high blank values; we have also found that it is not suitable for materials containing much organic matter as the platinum vessel employed for the fusion is attacked. The possibilities of a rapid method incorporating wet oxidation have therefore been examined.

Wet oxidation has been used for determining sulphur in pyrite, with, for example, a solution of bromine in carbon tetrachloride being used for the initial attack and then treatment with nitric acid[3]; a mixture of bromine and aqua regia has also been suggested.[4] Complete attack on materials containing silicates, organic matter and a resistant sulphide, such as pyrite, is possible by a combination of aqua regia with hydrofluoric and perchloric acids. Such an operation could not be conducted in a single stage in a glass or platinum vessel, but becomes possible if a basin of polytetrafluoroethylene, which is resistant to hydrofluoric acid, aqua regia and also to hot perchloric acid, is used. It has been found that the destruction of organic matter and pyrite by the oxidising mixture is expedited by adding vanadium pentoxide to the extent of 10 to 20 per cent. of the sample weight.

In the fusion method, leaching of the melt serves to remove iron and titanium, which would interfere in the gravimetric determination of sulphur as barium sulphate. In the wet-oxidation method, cupferron extraction provides a convenient way of removing these interfering elements.

A possible source of error associated with the use of perchloric acid is the loss of sulphate by volatilisation. It has been stated[2] that, provided evaporations are made at as low a temperature as possible, no loss of sulphate occurs in the presence of a sufficient excess of calcium salts. The use of polytetrafluoroethylene basins ensures a low temperature of evaporaton because of its low thermal conductivity, and our work shows that, provided there is a two-fold excess of calcium, no loss of sulphate sulphur occurs even after three evaporations with perchloric acid.

Barium, if present in a significant amount, would be expected to interfere by removing sulphur as barium sulphate if the solution is filtered before the final precipitation. To ascertain the amount of barium that could be tolerated, tests were carried out with various proportions of barium and sulphate. After one evaporation to dryness with 5 ml of 60 per cent. w/v perchloric acid, the residue was taken up in 3 ml of concentrated hydrochloric acid and, after dilution, the solution was filtered. Sulphate in the filtrate was precipitated in the usual way as barium sulphate. Table I shows the effect of barium originally present on the recovery of sulphate. For the average igneous rock in which barium rarely exceeds 0·2 per cent. of barium oxide and total sulphur 0·2 per cent., interference by barium will be negligible. Barium oxide (10 mg), equivalent to 1 per cent. on a 1-g sample of rock, caused a serious loss of sulphate, and recovery of a high sulphur content was significantly diminished by barium equivalent to 0·2 per cent. of barium oxide in the sample.

TABLE I

EFFECT OF BARIUM ON THE RECOVERY OF SULPHUR

Amount of barium oxide added, mg	Amount of sulphur added, mg	Amount of sulphur recovered in— barium sulphate residue, mg	filtrate, mg
10·0	10·0	2·05	7·95
2·0	10·0	0·18	9·84
0·0	10·0	0·00	10·00
10·0	2·0	1·02	0·93
2·0	2·0	0·00	1·98
0·0	2·0	0·00	1·99

METHOD

To 1·000 g of rock powder in a 3-inch polytetrafluoroethylene basin add a few millilitres of water to moisten the powder, and then, successively, 15 ml of concentrated nitric acid, 2 ml of concentrated hydrochloric acid and 10 ml of hydrofluoric acid. Place a polythene cover on the basin, and set aside overnight.

Uncover the basin, and evaporate the contents to dryness on an air bath. If the rock powder is completely decomposed, add a few millilitres of water and 10 ml of concentrated nitric acid, and again evaporate to dryness. Repeat this last operation once more. If decomposition is not complete after the first evaporation, add 5 ml of 60 per cent. perchloric acid and, if organic matter is present, 100 mg of vanadium pentoxide. In addition, if the material is low in calcium, add not less than 100 mg of calcium chloride. There should be present at least two equivalents of calcium for each equivalent of sulphur. Evaporate to dryness, and, if any organic matter or black mineral particles remain, evaporate with further portions of perchloric acid until decomposition is complete.

After the final evaporation, add 3 ml of concentrated hydrochloric acid and about 20 ml of water. Digest on the air bath for 15 minutes with occasional stirring, then cool and transfer the solution to a 250-ml separating funnel. Dilute to about 100 ml, and extract iron and titanium with successive 50-ml portions of a 1 per cent. w/v solution of cupferron in chloroform until the extract is no longer coloured brown. Wash the solution twice with 50-ml portions of chloroform, and finally with 50 ml of light petroleum. Run off the aqueous layer into a 400-ml beaker, and wash the light petroleum twice with 10-ml portions of water. Dilute the combined aqueous layer and washings to about 200 ml, and filter if necessary. Precipitate the sulphate as barium sulphate in the usual way. After ignition, the barium sulphate precipitate should be perfectly white. Blank values are usually insignificant.

RESULTS

The method was applied to samples of a number of rocks, including some containing a relatively large amount of barium, and the results, in comparison with those obtained by the fusion method, are shown in Table II.

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TABLE II

COMPARATIVE RESULTS BY THE WET OXIDATION AND FUSION METHODS

Rock type	Principal mode of occurrence of sulphur	Barium oxide content, %	Total sulphur found by—	
			fusion, %	wet oxidation, %
Camptonite	Pyrite	0·15	0·18	0·19
Altered quartz dolerite	Pyrite	0·18	0·36	0·39
Black ven marl	Pyrite, Sulphate	—	1·56	1·58
Quartz dolerite	Pyrite	—	0·17	0·16
Sillimanite - biotite - garnet schist..	Pyrite	—	1·03	1·04
Appinite - lamprophyre	Pyrite	—	0·32	0·34
Cummingtonite	Pyrite	0·11	0·17	0·18
Albite - hornblende - biotite schist..	Pyrite	—	0·05	0·05
Quartz-feldspar granulite	Pyrrhotite	—	0·46	0·43
Hornblendic Green Beds	Pyrite?	—	0·15	0·12
Shale	Pyrite, pyrrhotite, organic sulphur?	—	2·45	2·48
Shale		—	0·23	0·25
Shale		—	0·04	0·03
Shale		—	4·37	4·37

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The Volumetric Determination of Sulphur in Organic Compounds by Oxidation with Hypobromite Solution

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ATTEMPTS have been made to determine inorganic sulphides by oxidation to sulphate with standard bromate,[1] iodate[2] or bromine[3] solutions. Willard and Cake[4] pointed out several disadvantages in these methods; they used sodium hypobromite for the oxidation and found that sulphide was rapidly and completely oxidised to sulphate by the excess of hypobromite. By using this reaction Willard and Cake accurately determined sulphur in steels. Later, Erdey and Inczedy[5] determined sulphur in iron by titration with hydrogen peroxide and sodium hypobromite solution. Meulen[6] determined sulphur iodimetrically by reducing the organic sulphur to hydrogen sulphide; this was achieved by passing the vaporised organic compound with hydrogen through a platinum combustion tube containing platinum as a catalyst, and then absorbing the products in sodium hydroxide. Goswami and Sarkar[7] verified Meulen's procedure by determining sulphur in several organic compounds.

Batt[8] fused sulphur-containing organic compounds with sodium, and determined the sulphur gravimetrically; Zimmermann[9] used potassium instead of sodium for the fusion. However, it was found that, when the fusion was carried out with sodium or potassium, nitrogen in sulphur-containing organic compounds interfered owing to the formation of cyanide.[10]

All these methods require special equipment and rigid experimental conditions or they are insufficiently rapid. A simple, accurate and rapid procedure is described here for determining sulphur in non-volatile organic compounds by fusion with sodium in the presence of a mixture of sodium chloride or potassium carbonate, sodium carbonate and a reducing agent such as glucose; the fusion products are then oxidised with sodium hypobromite solution, and the excess of hypobromite is determined by titration with sodium thiosulphate solution. The interference owing to formation of cyanide during fusion of nitrogen-containing organic compounds has been overcome.

METHOD

REAGENTS—

All reagents were of recognised analytical grade.

Fusion mixture—A mixture of 7 parts of sodium chloride, 13 parts of sodium carbonate and 2 parts of glucose or a mixture of 5 parts of potassium carbonate, 5 parts of sodium carbonate and 1 part of glucose.

Sodium hypobromite solution—Prepared by dissolving 16 g of sodium hydroxide in 40 ml of water, to which 4 ml of bromine had been added, and diluting to 1 litre.[11] The solution was stored in an amber-coloured bottle at room temperature (20° to 40° C).

Potassium iodide solution, 10 per cent. w/v.

Sulphuric acid, 2 N.

Sodium thiosulphate, 0·1 N—Standardised against 0·1 N potassium iodate prepared from the reagent previously dried at 120° C.

PROCEDURE—

The accurately weighed organic compound (20 to 50 mg) was fused with sodium and 1 g of fusion mixture in a 5 to 6 inches long Pyrex-glass fusion tube for about 10 minutes, and the red-hot tube was then plunged into a beaker containing water. The solution obtained was warmed, and then filtered. The pieces of broken glass from the fusion tube were ground in a mortar and then washed, and the washings were added to the previous filtrate. To prevent atmospheric oxidation, the solution must not be heated, and it must be filtered through a filter-paper previously washed with water, otherwise inaccurate results will be obtained owing to the absorption of sulphide on the filter-paper.

Seventy-five millilitres of 0·1 N sodium hypobromite solution were added to the filtrate, and the flask was set aside for about 5 minutes. Twenty millilitres of 10 per cent. w/v potassium iodide solution and 50 ml of 2 N sulphuric acid were then added, and the liberated iodine was titrated against sodium thiosulphate with starch as indicator.

It was, however, found more convenient to adjust the filtrate, after fusion, to a known volume (100 ml) and to take a 10-ml aliquot of the filtered solution for the determination. The procedure was then the same as described above, except that only one-tenth of the stated volumes of sodium hypobromite, potassium iodide and sulphuric acid were used.

TABLE I

DETERMINATION OF SULPHUR IN DIFFERENT COMPOUNDS

Compound	Theoretical sulphur content, %	Sulphur found, %	Mean
Sulphosalicylic acid (hydrated)	12·58	12·45, 12·49, 12·50	12·48
Toluene-*p*-sulphonyl chloride	16·78	16·53, 16·46, 16·57	16·52
Sulphanilic acid (anhydrous)	18·49	18·35, 18·36, 18·31	18·34
Thiourea	42·10	42·55, 42·41, 41·85	42·27
DL-Cystine	26·63	26·36, 26·42, 26·27	26·35
Methionine	21·44	20·98, 21·04, 21·07	21·03
Methyl orange	9·79	9·59, 9·71, 9·77	9·69
Sulphanilamide	18·58	18·40, 18·36, 18·29	18·35
Sulphathiazole	25·10	25·34, 25·32, 25·33	25·33
Phenylthiourea	21·07	20·92, 20·82, 20·66	20·80
Sulphamic acid	33·03	32·88, 32·93, 32·89	32·90
Sulphaguanidine	13·79	13·75, 14·21, 14·31	14·09

RESULTS

The proposed method was used for determining sulphur in several sulphur-containing organic compounds. The purest available commercial compounds were used, and most of them were further purified by conventional methods. Each result was calculated from the average sodium thiosulphate titre from four titrations (1 ml of 0·1 N sodium thiosulphate ≡ 0·4 mg of sulphur) and three determinations were carried out for each compound. The results (see Table I) indicate that almost all the sulphur present could be accurately determined.

DISCUSSION OF THE METHOD

The proposed procedure provides a convenient method for determining sulphur in organic compounds, and does not involve any rigid experimental conditions; the time required for the determination is much less than by other procedures. The fusion mixture not only facilitates the complete reduction of organic sulphur to sulphide at low temperature, but also overcomes interference from organic nitrogen owing to the formation of hydrogen cyanide[12] or gaseous nitrogen,[13] which escape to atmosphere. The presence of glucose in the fusion mixture reduces the reduction time and converts the organic sulphur completely to sulphide.[14] Sodium chloride or potassium carbonate in the fusion mixture lowers the fusion temperature.

As sodium hypobromite solution is not completely stable,[11] any error owing to a change in concentration is avoided by using an excess of the solution in each titration.

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The Determination of Tantalum and Niobium in High-grade Minerals and Ores

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THE first successful technique for the accurate separation and determination of Ta_2O_5 and Nb_2O_5 was that devised and practised by Schoeller and Powell.[1] By carrying out the procedure they described, it is not a difficult task for the analyst to obtain the mixed oxides $(Ta, Nb)_2O_5$ in a reasonably pure state, but the separation of the one oxide from the other, despite the detailed instructions, is always a formidable task for those attempting it without the benefit of a demonstration by an expert.

Various procedures have been suggested as alternatives to the Schoeller and Powell oxalate - tannin procedure; these are mainly of three types, (*a*) chromatographic, (*b*) volumetric and (*c*) colorimetric. All these methods have been tried in the past, and each has its individual adherents. It had been hoped that atomic-absorption spectroscopy might have an application in this field, but investigators so far have failed to obtain any response from solutions of tantalum.

Chromatographic separation is commonly made on paper powder with the use of fluoride solutions and solvents such as ethyl acetate, ethyl methyl ketone or butyl methyl ketone. Several disadvantages have been found in applying this technique. It is essential to use polythene apparatus, and this has the well known properties of low heat transfer and proneness to contamination by absorption. Large volumes of organic solvents of low flash-point are used and must be removed by evaporation. In addition, considerable volumes of solutions containing hydrofluoric acid have to be handled. Volumetric methods have been adversely criticised in the literature. Of the colorimetric methods that have been suggested, three seem to have found more favour than the others, these being the thiocyanate method for determining niobium as described by Freund and Levitt[2]; Geld and Carrol's sulphuric acid - phosphoric acid - hydrogen peroxide method, also for determining niobium, which has been adapted by Pickup[3] and is described in two papers issued by the Geological Survey; the pyrogallol method of Hunt and Wells[4] of the National Chemical Laboratory.

Of these procedures, the thiocyanate method gives good results for determining niobium, but tantalum has to be determined by difference. In the hydrogen peroxide method the dependence of colour on time and temperature is not mentioned, and it has been found that, if optical-density readings are taken at 10 minute intervals, the variation is such as to invalidate the result. It has also been found that the colour is temperature sensitive. Further, the interference due to tantalum at 365 mμ is stated to be only 1 per cent., but it has been found that at the maximum sensitivity for niobium, 355 mμ, the interference increases to about 3 per cent. This method is not, therefore, entirely satisfactory.

The most attractive procedure is one in which pyrogallol is used, since both niobium and tantalum can be determined, and, although each interferes to some extent with the determination of the other, this interference can be calculated and allowed for as is, in fact, suggested by Hunt and Wells.

Their method has been investigated by Catoggio and Rogers,[5] who have found that sensitivity can be increased by the presence of Ethoquad (polyethoxyl-15-stearyl-methylammonium chloride), and that the complex can be extracted with ethyl acetate. Under these conditions, lower interference values were obtained for niobium in the presence of tantalum, but higher values for tantalum in the presence of niobium. For the purpose of this note the original Hunt and Wells method has been used.

It has been found that the pyrogallol colour is completely stable for several hours and even overnight, giving ample time for measuring the optical density. It was thought that the calculation of mutual interference could be dispensed with if calibration graphs were made by using, as standards, mixed oxides of niobium and tantalum in known ratios. With this in view, calibration graphs were prepared at both the niobium and tantalum wavelengths from solutions containing 0 to 50 mg of Nb_2O_5 and 50 to 0 mg of Ta_2O_5 in 10-mg increments, the oxides being fused with potassium pyrosulphate and leached with ammonium oxalate as described below. It was found that from these graphs both tantalum and niobium could be accurately determined. Fifty milligrams of mixed oxides were found to be sufficient for the determinations; if this amount is not available from the products of the analysis, it is possible to work with 10 mg or, alternatively, fixed amounts of one or the other pure oxide can be added to the mixed oxides from the analysis. By using this technique the time necessary for an analysis of a tantalite or columbite can be considerably shortened. The proposed procedure is based on Schoeller and Powell's method.

METHOD

REAGENTS—

Potassium pyrosulphate—Analytical-reagent grade.

Tartaric acid, 20 per cent. w/v.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

Cupferron solution, 6 per cent. w/v.

Sulphuric acid, 5 per cent. v/v.

Tannic acid.

Ammonium oxalate—Analytical-reagent grade.

Potassium bisulphate solution, 10 per cent. w/v.

Alkaline pyrogallol solution—Dissolve 1 g of pyrogallol in 30 ml of 20 per cent. sodium sulphite solution, and filter into a 50-ml calibrated flask. Wash any residue, add the washings to the contents of the flask, and make up to the mark with 20 per cent. sodium sulphite solution.

Acid pyrogallol solution—Dissolve 10 g of pyrogallol in 35 ml of water and 2·5 ml of sulphuric acid (1 + 1), and filter into a 50-ml calibrated flask. Wash any residue, add the washings to the contents of the flask, and make up to the mark with water.

PROCEDURE—

Fuse 0·5 g of finely ground sample with 5 g of potassium pyrosulphate in a silica crucible, cool, and extract the cooled melt with 30 ml of 20 per cent. tartaric acid solution. Filter the solution, and wash and ignite the residue. If necessary, treat the residue in a similar way to the original melt, using smaller amounts of reagents, and combine the solutions. Add 30 ml of concentrated hydrochloric acid to the solution (200 ml), boil for 2 to 3 minutes, and stir in paper pulp; filter off the precipitate, wash, and ignite it. Leach the mixed oxides with hydrochloric acid, filter, and re-ignite before weighing as major earth-acid oxides. Add cupferron solution to the combined filtrates from the hydrochloric acid hydrolysis, and filter; wash, and ignite the precipitate, which contains minor amounts of earth acids with iron, titanium, vanadium, etc. Fuse the ignited residue with 3 g of potassium pyrosulphate, and leach the cooled melt with 50 ml of 5 per cent. sulphuric acid containing 2 per cent. of tannin. Boil the solution for a few minutes, stir in paper pulp, allow to cool, and then filter; wash the precipitate with 5 per cent. sulphuric acid, and ignite to give the minor fraction of earth-acid oxides. Weigh, and grind the two fractions together in a small agate mortar, weigh out 50 mg into a silica crucible, and fuse with 1·5 g of potassium pyrosulphate. Leach the cooled melt with a solution of 2 g of ammonium oxalate, and make up to exactly 50 ml.

For the determination of niobium, transfer 1 ml of this solution, by pipette, to a 50-ml calibrated flask, add 20 ml of 4 per cent. ammonium oxalate solution, 5 ml of 10 per cent. potassium bisulphate solution and 20 ml of freshly prepared alkaline pyrogallol solution, dilute to the mark with water, and measure the optical density at 410 mμ in a 1-cm cell after 10 minutes. Determine the amount of niobium present from the calibration graph.

For the determination of tantalum, transfer by pipette 5 ml of the solution prepared as described above to a 25-ml calibrated flask, and add 4 ml of 10 per cent. potassium bisulphate solution and 0·8 g of powdered ammonium oxalate. Dilute the mixture to about 20 ml, shake until solution is complete, add 2 ml of acid pyrogallol solution, and then make up the solution to 25 ml. Determine the optical density in a 1-cm cell at 400 mμ, and determine tantalum by reference to the appropriate calibration graph.

In both determinations a reagent blank reading must be taken.

If the material being analysed contains an excessively large amount of iron, this can be removed, before the cupferron precipitation, with hydrogen sulphide after the acid solution has been neutralised with ammonium hydroxide solution. If the presence of tungsten is suspected, the magnesia separation described by Schoeller and Powell can be carried out on the combined mixed oxides before proceeding to the colorimetric determination.

The amount of titanium dioxide present in tantalite or columbite is usually small enough to have been eliminated by the procedure described, but its absence can be checked by a colorimetric test on part of the mixed oxides obtained by Schoeller and Powell's procedure. If any titanium dioxide is present, the mixed oxides may be subjected to a further pyrosulphate - tannin extraction before continuing with the colorimetric procedure, but it is usually much simpler and more convenient to add to the standard mixture the equivalent of any titanium dioxide found.

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A Conductimetric Adaptor for a Recording Titrator

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After constructing a recording potentiometric titrator based on the design prepared by Irving and Petit,[1] it was decided to extend the scope of the instrument by means of an adaptor to include conductimetric titrations. One of the main virtues of Irving and Petit's design was the ability to use either the recorder or, more particularly, the pH meter for uses other than recording a titration, with a minimum of alteration. It was therefore thought that the components needed for conductimetric titrations would most conveniently be assembled in a small unit that could be quickly plugged into the main instrument when needed, and stored away when not in use.

One of the simplest methods of measuring the conductivity of a solution is to measure directly the current passing through the solution at a fixed alternating potential or to measure the potential developed across a standard resistance in series with the cell and the applied potential. The potential across the resistance is then directly proportional to the conductivity of the solution, and therefore is in a form suitable for display on a potentiometric recorder.

Colvin and Propst[2] have described such an instrument in which an a.c. signal from a power oscillator is fed in series with the conductivity cell and a standard resistance. The potential developed across the resistance is rectified and applied to one terminal of a 10-mV recorder. Part of the signal from the oscillator is rectified and applied, via a Helipot, to the other side of the recorder, thus supplying zero suppression.

It was decided to follow this circuit basically, but with the simplification of using 50-cycle a.c. derived from the mains via a stepdown transformer. By working at mains frequency, it is possible to use synchronous choppers for the rectification, thus assuring linearity of the conductance scale, which is not easily achieved with other methods of rectification at the millivolt level. Zero suppression is obtained from a battery circuit to ensure adequate stability.

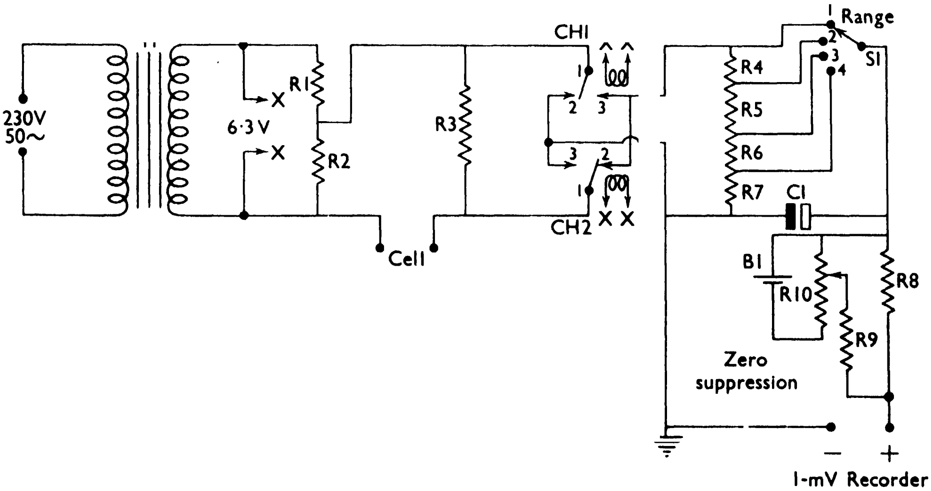

Fig. 1. Circuit diagram of adaptor (for values of components, see Appendix, p. 147)

Description of the instrument

The circuit of the adaptor is shown in Fig. 1. The mains supply, via a constant-voltage transformer if necessary, is fed to a valve-heater transformer, which produces a secondary voltage of 6·3. This potential is applied across a divider consisting of two 5-ohm 4-watt resistors in series. The 3·15 volts developed across one of the resistors is applied to the conductivity cell in series with a 10-ohm high-stability resistor. The potential developed across this resistor by current flowing in the cell circuit is rectified by two A.E.I. synchronous choppers, type CK3, wired in the manner shown, which produce full-wave rectification. The coils of the choppers are supplied with 6·3 volts from the same transformer. The rectified potential is then applied across a resistor chain connected to a range switch to produce four recorder full-scale conductivity ranges of approximately 50, 250, 1000 and 5000 μmho. These ranges are somewhat arbitrary, but were thought to be satisfactory for most of the work envisaged for the instrument. The output from the range switch is smoothed by a large capacity electrolytic capacitor and then taken to the positive terminal of a 1-mV recorder, via the zero suppression circuit. This provides a potential of up to three times the full-scale deflection of the recorder in the opposite direction to the signal derived from the conductivity cell. The negative side of the circuit goes directly to the recorder negative and is earthed.

The components for the adaptor were housed in a small case, 9 inches × 5 inches × 5 inches high. A Mullard dip-type conductivity cell is used, the leads of which plug into the front panel. The wiring of the instrument is simple, but some care is needed to connect the choppers and their coils in the right sense.

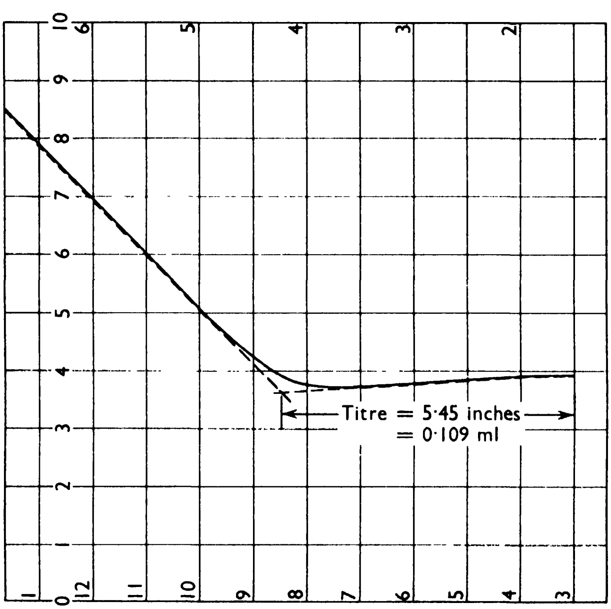

Fig. 2. Titration of 1 ml of approximately 1 per cent. ammonium maleate solution with 0·05 N iodic acid

The pin numbering is given in the circuit diagram, but the coil connections are plugged into the top of the choppers with the special cap supplied. The wires attached to the top cap are coloured red and black, and in connecting these to the 6·3-volt supply, the same coloured wires from each cap must be attached to the same side of the supply. It still remains to decide which side of the supply to connect the red and black connections, as this will decide the polarity of the rectified signal, but this is most easily found by practical trial.

It was found that, if zero suppression was not used, a constant-voltage transformer was not necessary to obtain satisfactory results, but it was found to be essential when any significant degree of suppression was in use. At maximum zero suppression, even with the constant-voltage transformer, the trace was rather erratic, but straight lines could be interpolated on the chart.

To put the adaptor into use, the output is plugged into the recorder pen circuit, in place of the pH meter, the conductivity cell is placed in the titration assembly and the leads are plugged into the front panel of the adaptor.

If examination of a titration curve shows a large background conductivity, with only a small change occurring during the titration, the titration may be repeated and the zero suppression control advanced at the start of the titration to remove all or part of the background conductivity reading, *i.e.*, the initial recorder reading may be partly or completely cancelled out. The instrument can then be switched to a more sensitive range, thus magnifying the change in conductivity resulting from the titration. The degree to which zero suppression is used must depend largely on the type of titration.

Results and discussion

The adaptor has been used for carrying out conductimetric titrations between strong acid and strong base, strong acid and salt of a weak acid and some precipitation reactions, *e.g.*, titration of sulphate with barium acetate solution. In all titrations for which conductivity is proportional to concentration, straight line plots have been obtained. An example of a replacement reaction, the titration of ammonium maleate with standard iodic acid, is shown in Fig. 2. Iodic acid was used in preference to hydrochloric or sulphuric acids because the iodate ion has a much lower mobility than the chloride or sulphate ions, comparable in fact to the acetate ion.

The accuracy of the titration obviously depends to a large extent on the system under examination, but in favourable instances it should be determined by the accuracy of the recording titrator, about 100 ± 0·5 per cent. on a titration occupying 10 inches of chart.

Appendix

List of components for the adaptor

(Fig. 1)

R_1, R_2 = 5-ohm 3-watt resistors.
R_3, R_8 = 10-ohm ½-watt high-stability resistors.
R_4 = 820-ohm ½-watt resistor.
R_5 = 150-ohm ½-watt resistor.
R_6 = 39-ohm ½-watt resistor.
R_7 = 10 ohm ½-watt resistor.
R_9 = 4700-ohm ½-watt resistor.
R_{10} = 1000-ohm wire-wound variable resistor.
C_1 = 1000-μF 6-volt electrolytic capacitor.
S_1 = 4-way 1-pole rotary switch.
B_1 = 1·5-volt dry cell
CH_1, CH_2 = Synchronous choppers, A.E.I. type CK 3.
T_1 = Transformer : input 230 volts; output 6·3 volts at 2 amps.
Constant-voltage transformer, if required: Advance CVH 60A.

I acknowledge the help of J. R. Martin in various aspects of the design, J. F. Burbridge for constructing the instrument and the directors of the Distillers Company Ltd. for permission to publish this paper.

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Received *August* 14*th*, 1962

Collection of Gas-chromatographic Fractions for Identification by Infrared Spectrophotometry

By D. A. SHEARER, B. C. STONE

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Although many types of apparatus are available commercially and many more have been proposed for the collection of gas-chromatographic fractions, they are mainly useful only on a milligram scale, *e.g.*, Haslam, Jeffs and Willis[1] have decribed methods that will handle down to 1 to 2 μl of liquids. However, when 0·2 mg or less of material is to be collected, difficulties are encountered, the main one being that, with the rapid flow of carrier gas from the outlet of the chromatograph, the fraction tends to be swept right through the trap. There is also the difficulty of transferring these small amounts to an infrared cell without prohibitive losses.

To overcome these difficulties we have carried out the collection in two stages. The fraction was first trapped in a short, packed column somewhat similar to that used by Farrington *et al.*,[2] and then transferred with a slow stream of nitrogen from the column to a trap, from which it was transferred to the infrared cell. The trap was designed in a way such that the collected sample could be picked up with a standard microsyringe.

APPARATUS

The gas chromatograph used was a Perkin-Elmer 154C, fitted with a hydrogen-flame ionisation detector. The effluent from the column (4½ feet × ¼ inch) packed with 10 per cent. Carbowax 1500 on 150- to 160-mesh Anakrom U) was split with approximately 1 per cent. going to the detector and the remainder to the collection column. The infrared analyses were carried out on a Perkin-Elmer 21 infrared spectrophotometer fitted with a lens-type beam condenser.

The collection column consisted of a 12-inch × ⅛-inch copper (or stainless steel) tube to which was silver soldered a Luer fitting (for direct connection to the sample outlet of the Perkin-Elmer 154C). The tube was enclosed in a ¼-inch copper tube by Swagelok heat-exchanger fittings. The details of construction are shown in Fig. 1. The outer tubing was insulated with a layer of asbestos tape for easier handling. The ⅛-inch inside tube was filled with uncoated 60- to 80-mesh Celite held in place with plugs of glass-wool.

The small trap was constructed of glass, as shown in the figure, and was approximately 2 inches long. A length of 20-gauge hypodermic needle, which had a bore sufficiently large to allow insertion of a 10-µl Hamilton syringe, was held in place inside the trap with a piece of rubber stopper; the connecting sleeve was also rubber.

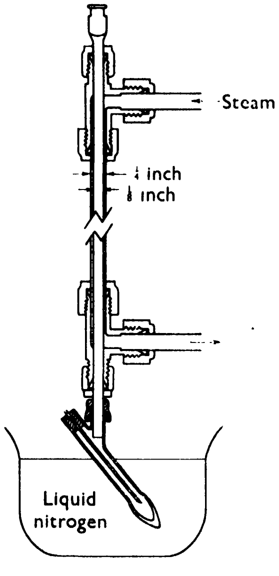

Fig. 1. Fraction collector

PROCEDURE

Cold water is passed through the outer jacket of the heat exchanger, and the metal trap is connected to the outlet of the gas chromatograph so as to collect the desired fraction. The small glass trap can be left in place during this step if desired. This will prevent condensation of moisture on the end of the tube that fits into the glass trap. After the fraction has been collected the apparatus is assembled as shown in Fig. 1, and the glass trap is cooled in a bath of liquid nitrogen or simply in powdered dry ice. It is not recommended that a solvent be used with the dry ice because of the possibility of contamination of the sample with solvent. Steam is then passed through the outer jacket as shown, and a slow stream (2 to 3 ml per minute) of dry nitrogen is passed so as to carry the entrapped fraction from the Celite packing into the glass trap. This may take from 5 to 50 minutes, depending on the volatility of the fraction. The glass trap is then warmed, removed, and the walls are washed down with approximately 3 μl of solvent (*e.g.*, carbon disulphide). The glass trap is then placed in a 15-ml centrifuge tube, and the resulting solution is collected in the bottom tip by spinning in a centrifuge. The needle of a Hamilton microsyringe is then inserted to the bottom, and the solution is removed and transferred to an ultramicro cavity cell (Connecticut Instrument Corporation) or similar micro infrared cell.

RESULTS

Samples of isopentyl acetate, 2-octanone, and geraniol[3] were all successfully collected. Recoveries from 0·2-mg samples were approximately 50 per cent., based on the infrared curves.

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Received *September 3rd*, 1962

Book Reviews

THE ELECTRONICS OF LABORATORY AND PROCESS INSTRUMENTS. By V. S. GRIFFITHS, Ph.D., F.R.I.C., and W. H. LEE, Ph.D., F.R.I.C. Pp. xiii + 368. London: Chatto and Windus. 1962. Price 50s.

With the increasing use of electronic equipment in instruments designed for analytical purposes, it is almost essential that the analyst has at least a basic knowledge of the electronic principles involved. This is particularly important in small laboratories where the analyst cannot readily call upon the services of an expert. In large organisations the electronics engineer is available when instrumental faults occur; nevertheless, valuable time can often be saved if the operator has sufficient knowledge to be able to detect and rectify even simple faults.

This book has not been written for the electronics expert, but is clearly intended for anyone with a background in general physics wishing to gain a working knowledge of the operation of certain instruments in general use in chemical laboratories, in particular. Fortified with this information the analyst will be the better able to use such instruments more intelligently, have a better appreciation of their limitations and be in a position to make simple modifications and possibly suggest improvements to the manufacturers.

The first part of the book reviews the behaviour of resistances, and of inductances under direct and alternating voltages, and emphasises the more practical importance of resonance. The principles of thermionic emission are considered and various types of valves and photo-cells are described. The authors deal, in a general way, with the application of these valves and with such ancillary devices as metal rectifiers and vibrators, under the headings rectification, amplification and oscillation.

The second part deals with typical laboratory equipment, all of which is of special interest to the analyst, for example, pH meters, spectrophotometers and polarographs; a useful chapter on radioactivity measurement is also included. W. T. ELWELL

PRACTICAL CLINICAL BIOCHEMISTRY. By HAROLD VARLEY, M.Sc., F.R.I.C. Third Edition. Pp. viii + 689. London: William Heinemann Medical Books Ltd.; New York: Interscience Books Inc. 1962. Price 50s.

When I reviewed the second edition of this book some 2 years ago I said that I thought it was one of the best laboratory text-books for practical workers in clinical laboratories. Further experience has shown that this volume is used for reference more than any other book on the subject; at least this is so in my laboratory.

The new edition retains all that was in the previous volume, but contains a number of extremely useful additions, the most important of which is the chapter on acid - base regulation, which has been augmented to include the Astrup technique and Campbell and Howell's re-breathing method for determining P_{CO_2}.

The chapter on hormones has been revised and enlarged, and the methods for determining adrenaline and noradrenaline have been brought up to date to include the methylated amines. In addition, there is a technique given for mandelic acid metabolite.

Scattered throughout the book it is seen that there are several other new methods included, as, for instance, the glucose oxidase test for blood glucose, the diacetyl monoxime method for urea and other nitrogenous substances, and the D-xylose excretion test.

The chief feature of this edition is a chapter on enzymes, in which several of the newer methods have been grouped together. These include enzyme tests for blood pyruvate and lactate that did not appear in the previous volume. Of interest also to practical workers is the method for carrying out cortisone stressed glucose tolerance tests, and for those working on the sterols a method is given for the determination of pregnanetriol.

These new additions have extended the scope of a book that was already much more comprehensive than any other similar volume on clinical biochemistry, and I am sure that it will continue to be used widely. R. F. MILTON

SPECTROSCOPY. Report of the Conference organised by the Hydrocarbon Research Group of the Institute of Petroleum and held in London, March, 1962. Edited by M. J. WELLS. Pp. viii + 305. Oxford, London, New York and Paris: Pergamon Press, distributors for The Institute of Petroleum. 1962. Price 63s.

This is a collection of 23 papers on a wide range of spectroscopic topics. Two are of more general interest than the others. The first, by H. W. Thompson, is entitled "Trends in the Chemical Applications of Spectroscopy," and although it occupies only 12 pages it manages to cover a great deal of ground in a readable and informative manner. Van Zandt Williams of the Perkin-Elmer Corporation contributes an even shorter article on developments in applied spectroscopy, looking primarily at trends in instrumentation. He uses the work "applied" to denote "the total use of a spectroscopic principle and not just the narrow first wave of research." In molecular spectroscopy, quantitative analysis is characteristic of ultraviolet and mass spectroscopy and X-ray diffraction. Qualitative analysis predominates in methods such as infrared, nuclear magnetic resonance and electron paramagnetic resonance spectroscopy. Williams traces the transition from specialist exponents of a single spectroscopic field (*e.g.*, infrared spectroscopists) to "generalist" users of later and somewhat simplified instruments capable of coping with a large proportion of the work done with more complex apparatus. The trend in mass spectroscopy reflects powerful competition from vapour chromatography, and infrared spectroscopy is "on a plateau," with n.m.r. reinforcing it and sometimes supplanting it. Williams sees possibilities of great advances by the use of digital output and computers, but they will come slowly because "much of today's data is too inaccurate." This is an interesting essay with its own flavour, as in the following sentence: "Just as hired help has disappeared from the home in the last generation, so the technician is disappearing from the analytical laboratory."

Among the other papers is a concise and authoritative account of atomic-absorption spectroscopy by A. Walsh and a specialised contribution on the same theme by R. L. Warren. H. N. Wilson and R. J. Otter discuss X-ray fluorescence analysis, "the most powerful technique for elemental analysis that has been developed in the last ten years."

Nuclear magnetic resonance is ably introduced by R. E. Richards, and R. B. Williams contributes a substantial study of n.m.r. performance parameters, which is suitable for expert workers.

Electron resonance principles, methods and techniques, are explained in some detail by D. J. E. Ingram and examples are given of applications to metal complexes of organic compounds. D. H. Whiffen contributes a paper on electron spin resonance of free radicals, and R. N. Dixon gives an account of methods for observing electronic spectra of free radicals and other unstable species. T. M. Sugden reviews the spectrophotometry of free radicals in flames and G. W. Chantry discusses optical absorption spectra of oriented free radicals. The two last mentioned papers show faulty proof reading and numbering of diagrams, which holds up the unsuspecting reader.

An interesting article on far infrared spectroscopy (G. R. Williams, S. A. Inglis and C. Smart) explains why this region of the spectrum is not particularly fruitful for analytical chemistry.

A paper on rotation in solution by W. J. Jones and N. Sheppard is followed by a longer article by L. J. Bellamy on the origins of group frequency shifts. This is an able and somewhat speculative treatment of the influence of inductive effects and of non-bonded interactions on infrared group frequencies, but as a piece of exposition it suffers from over-compression.

A discussion by H. W. Thompson of factors affecting the characteristics of infrared and Raman vibrational bands conducts the reader skilfully through an intricate story. Solvent effects in infrared group frequencies are neatly reviewed by H. E. Hallam and the theme is pursued for inorganic complex hydrides, carbonyls and nitrosyls by D. M. Adams.

W. C. Price, R. Bralsford and D. M. Roessler give examples of ultraviolet spectra in the "vacuum" region where photoionisation of molecules and radicals can occur.

R. K. Ritchie, A. D. Walsh and P. A. Warsop discuss the electronic spectra of small polyatomic molecules with special reference to the absorption spectrum of nitrogen dioxide.

There is a short and not very optimistic article by A. D. Booth on some uses of electronic computing machines in spectroscopy.

The book suffers a little from its heterogeneity and from the varying demands made by different authors on the reader's previous knowledge. Assumptions appropriate for a Conference audience may flatter the wider scientific public but delay comprehension. The volume is, however, at this time important because it aims at the "generalist" user of spectroscopic methods as much as at the narrow specialist. Much of the exposition is admirable, at a level somewhere between that of the text-book and the original paper.

Publication of this kind of conference report is justified if the papers reach a wide audience quickly. To publish in July a full account of a March Conference is a fine achievement and librarians should get the book into circulation while its impact is greatest. R. A. MORTON

CHROMATOGRAPHIC REVIEWS: PROGRESS IN CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS. Volume 4. Edited by MICHAEL LEDERER. Pp. viii + 184. Amsterdam and New York: Elsevier Publishing Company. 1962. Price Dfl. 25.

Chromatographic Reviews is becoming increasingly independent of the *Journal of Chromatography*. Four of the 8 reviews in this 4th volume, occupying well over half the total space, represent previously unpublished material. Two others are reprinted, and the remaining two are translations into English from the Journal. The usual high standard has been maintained by all the authors; their reviews are crisply written and packed with well documented information. The first, by Pocchiari and Rossi, covers the techniques of paper chromatography of radioactive substances, in particular automatic scanning devices for one-dimensional and two-dimensional chromatograms. This is usefully supplemented by Adloff's brief paper on gas chromatography of radioactive substances. Various arrangements are described for discontinuous or continuous simultaneous recording of active and inactive peaks. The power of these methods is illustrated by the detection of 20 radioactive species in neutron-irradiated n-propyl bromide.

Thin-layer chromatography is brought up to date by Demole from his review in Volume 1. In view of its speed, sensitivity and wide applicability to either adsorption or partition methods, one is inclined to wonder why this technique has not displaced all others for analytical chromatography.

Knight has provided two long papers on chromatographic media, namely, ordinary and cation-exchange papers, for fractionation of amino acid mixtures. They record much of the author's own work relating resolution to grade of paper and technique, in conjunction with various solvent systems. The relatively new field of ion-exchange papers developed with aqueous buffers is thoroughly explored and appears valuable, especially since normal chromatography can be done on the same sheet in a second direction.

Bailey and Pridham discuss separation of oligosaccharides on columns and on paper by chromatography and electrophoresis. Data are tabulated for about 50 developing solvents and many reagents for localisation of different sugars, with 153 references.

Then comes a comprehensive review on porphyrins by Falk, which starts with methods for extraction and preparation of free and metal porphyrins and their esters. The merits of numerous exotic solvent systems are discussed, including a two-dimensional scheme involving reverse-phase chromatography in the second direction. Loads must be under 1 μg to avoid spreading, but less than 1/100 μg can be detected by fluorescence. Column chromatography and electrophoresis are also reviewed.

After 3 papers from Great Britain and one each from Italy, France, Switzerland and Australia, the volume concludes with a review from India on fractionation of higher fatty acids by reversed-phase paper chromatography and its application to natural fats.

The book is well produced, commendably free from errors and, like its predecessors, an invaluable reference work. E. LESTER SMITH

Publications Received

ROCK-FORMING MINERALS. Volume 2. CHAIN SILICATES. By W. A. DEER, M.Sc., Ph.D., F.G.S., R. A. HOWIE, M.A., Ph.D., F.G.S., and J. ZUSSMAN, M.A., Ph.D., F.Inst.P. Pp. x + 379. London: Longmans, Green and Co. Ltd. 1962. Price 95s.

LES COSMÉTIQUES: PHARMACOLOGIE ET BIOLOGIE. By HENRI THIERS. Pp. viii + 300. Paris: Masson et Cie. 1962. Price 65 NF.

THE ANALYTICAL CHEMISTRY OF INDIUM. By A. I. BUSEV. Translated from the Russian by J. T. Greaves. Pp. xvi + 288. Oxford, London, New York and Paris: Pergamon Press. 1962. Price 84s.

ANALYTIK DES KALIUMS: KLASSISCHE UND MODERNE TRENNUNGS- UND BESTIMMUNGSMETHODEN MIT LEISTUNGSVERGLEICHEN IN KRITISCHER SICHT. By HANS TOLLERT. Pp. xvi + 416. Stuttgart: Ferdinand Enke Verlag. 1962. Price (paper) DM 75; (cloth boards) DM 79.

RESIDUE REVIEWS: RESIDUES OF PESTICIDES AND OTHER FOREIGN CHEMICALS IN FOODS AND FEEDS. Volume 1. Edited by FRANCIS A. GUNTHER. Pp. iv + 162. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 22.

STANDARD METHODS FOR TESTING TAR AND ITS PRODUCTS. Edited by P. V. WATKINS. Fifth Edition. Pp. viii + 608. Gomersal, Leeds: Standardization of Tar Products Tests Committee. 1962. Price 63s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, 1962–1963. Pp. viii + 317. London: The Pharmaceutical Press. 1962. Price 20s.

KOLORIMETRIE, PHOTOMETRIE UND SPEKTROMETRIE: EINE ANLEITUNG ZUR AUSFÜHRUNG VON ABSORPTIONS-, EMISSIONS, FLUORESCENZ-, STREUUNGS-, TRÜNBUNGS- UND REFLEXIONSMESSUNGEN. By GUSTAV KORTÜM. Pp. viii + 464. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 48.

ADVANCES IN FLUORINE CHEMISTRY. Volume 3. Edited by M. STACEY, F.R.S., J. C. TATLOW, Ph.D., D.Sc., and A. G. SHARP, M.A., Ph.D. Pp. vi + 281. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 50s.

BIOLOGICAL TRANSPORT. By HALVOR N. CHRISTENSEN. Pp. viii + 133. New York: W. A. Benjamin Inc. 1962. Price $6.50.

HANDBOOK OF THERMOPHYSICAL PROPERTIES OF SOLID MATERIALS. Volume III. CERAMICS. Edited by ALEXANDER GOLDSMITH, THOMAS E. WATERMAN and HARRY J. HIRSCHHORN. Pp. viii + 1162. Oxford, London, New York and Paris: Pergamon Press. 1961. Price (per set of 5 volumes) £33.

Erratum

JANUARY (1963) ISSUE, p. 67, 23rd line from bottom. *For* "0·7 mg" *read* "0·07 mg.

MARCH, 1963 Vol. 88, No. 1044

THE ANALYST

EDITORIAL

Accent on Youth

It has always been the policy of the Society to encourage the reading of papers and to promote discussion of subjects of analytical interest. Speakers are drawn from far and wide. Some come from industrial laboratories, some from academic institutions or municipal undertakings; some are in private practice, some are in Government service. Many are acknowledged experts in their respective fields, others are at the beginnings of their careers—the experts of tomorrow. All have a common interest in some branch of Analytical Chemistry in its widest sense.

Addressing a public scientific meeting for the first time is an occasion of some moment for the young researcher. How will his ideas be received by an audience that almost certainly will include "elder statesmen" of the profession—scientists of far wider experience than his own? Is he perhaps a little apprehensive, for we find that the post-graduate student is not always as ready as we would wish to come forward spontaneously and describe his work. Yet there is no doubt that it is from the work of these young men and women, in the Universities and Technical Colleges throughout the country, that many of the most valuable advances will come and from whom many of the new techniques of the future will arise.

The Society is very conscious of the importance of the work being done at the Universities and Technical Colleges and the grade of "Junior Membership" was inaugurated some years ago so that the "younger generation" should be better represented in the Society's ranks. But there is still a reluctance, or a reticence, on the part of many Junior Members and their contemporaries to present papers at our meetings, and the Programmes Committee has recently been making strenuous efforts to break down this resistance. We should like at least one of our meetings each year to be set aside as a forum for discussion of work in progress at these centres of learning and thus to foster the active participation of the post-graduate students, who will, after all, be the practising analysts of the future.

We have been in touch with several Universities and Colleges of Technology and, broadly speaking, found them in sympathy with our views. With their help we have been able to arrange a meeting, to be held on April 9th at Chelsea College of Science and Technology, London, S.W.3, for which the programme will consist of six papers contributed entirely by these "up and coming" analysts. We look forward to hearing what they have to say and we are confident that an interesting and stimulating evening is in store.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

Ordinary Members elected January 9th, 1963

William Boardman, M.Sc.(Lond.), A.R.I.C., A.R.T.C.S.; Kenneth Roy Capper, B.Pharm., Ph.D., D.I.C.; Leslie Richard Chislett, A.R.I.C.; Hugh Bernard Clarke, M.Sc.(Lond.), Dino Coppini, L.D.; Sven Lars Ake Danielsson, Fil.lic.(Stockholm); Kenneth Wallace De Witt, B.Sc.(Bris.); Christopher John Dodd; Peter James Duff, B.Sc.(Liv.); John Henry Dunn, B.Sc.(Lond.), A.R.I.C.; Philip Sidney Hetherington, B.Sc.(Manc.), A.R.I.C.; John Douglas Hobson, B.Sc., Ph.D.(Lond.), F.R.I.C., F.I.M., A.Met.(Sheff.); David Francis James; David Peter Lones, B.Sc., Ph.D.(Birm.); R. C. Madan, B.Sc.; Benjamin Max Milwidsky, B.Sc. (Rhodes); John Michael Orme, B.Sc.(Lond.); Philip Geoffrey Quartermain, B.Sc.(Lond.), A.R.I.C.; Dennis Noel Raine, B.Sc., Ph.D., M.B., B.S.(Dunelm.); Cyril Redshaw, B.Sc. (Lond.), D.L.C.(Loughborough); Trevor Salvage; Paschoal Senise, D.Sc.(Sao Paulo); Graham Robert Thomas, A.R.I.C.; Eric George Towndrow, B.Sc.(Lond.), A.R.I.C.; Terry Wallis; Joseph Westheimer, B.S.(New York).

Junior Members elected January 9th, 1963

James Roger Cousin, B.Sc.(Hull); Michael Thompson, B.Sc.(Lond.), A.R.C.S., A.R.I.C.

Ordinary Members elected February 6th, 1963

James Haythorn Baird, B.A.(Rutgers), M.Sc.(Brooklyn); John Alexander Baynes, B.Sc. (Southampton); Ronald Francis Bird, A.R.I.C.; Lyn Davies, B.Sc.(Wales); James Sidney Double; Andrew Gordon Gavin, A.H.-W.C., A.R.I.C., A.M.Inst.F.; John Harry Greaves, B.Sc.(Lond.), F.R.I.C.; Geoffrey James Holland, B.Sc.(Lond.), F.R.I.C.; George Harold Jeffery, B.Sc., Ph.D.(Lond.), F.R.I.C.; William Arthur Lloyd; David Lyn Mack, L.R.I.C.; Gordon Nelson, A.R.I.C.; Ronald Platt; Bryan Edward George Pledger, B.Sc.(Lond.); Richard John Marshall Ratcliffe, B.Sc.(Lond.); Gerard Aloysius Rimmer, B.Sc.(Liv.), A.R.I.C.; Alan Sydney Waterhouse, B.Sc.(Lond.), A.R.I.C.; Glynne Williams.

NORTH OF ENGLAND SECTION

The thirty-eighth Annual General Meeting of the Section was held at 2.30 p.m. on Saturday, January 26th, 1963, at the Old Nag's Head Hotel, Lloyd Street, Manchester, 3. The Chair was taken by the Chairman of the Section, Mr. J. Markland, B.Sc., F.R.I.C. The following appointments were made for the ensuing year: *Chairman*—Mr. C. J. House. *Vice-Chairman*—Mr. J. F. Clark. *Hon. Secretary and Treasurer*—Mr. G. F. Longman, Unilever Research Laboratory, Port Sunlight, Cheshire. *Members of Committee*—Mr. C. E. Davis, Dr. J. R. Edisbury, Mr. B. Hulme, Mr. A. Hutchinson, Professor H. M. N. H. Irving and Mr. A. N. Leather. Mr. A. A. D. Comrie and Mr. F. Dixon were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of Section at which Mr. J. Markland, B.Sc., F.R.I.C., gave his Address as retiring Chairman. The Chair at this meeting was taken by the new Chairman of the Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

SCOTTISH SECTION

The twenty-eighth Annual General Meeting of the Section was held at 1.45 p.m. on Friday, January 25th, 1963, at More's Hotel, India Street, Glasgow, C.2. The Chair was taken by the Vice-Chairman of the Section, Dr. R. A. Chalmers, B.Sc. The following officer bearers were elected for the forthcoming year: *Chairman*—Dr. R. A. Chalmers. *Vice-Chairman*—Mr. J. K. McLellan. *Hon. Secretary and Treasurer*—Mr. J. W. Murfin, Standards Department, Boots Pure Drug Co. Ltd., Motherwell Street, Airdrie, Lanarkshire. *Members of Committee*—Dr. D. M. W. Anderson, Mrs. D. A. Edmond, Mr. J. C. Jack, Mr. W. J. Murray, Dr. J. Sandilands and Mr. A. F. Williams. Mr. J. S. Foster and Mr. R. A. Sutter were re-appointed as Hon. Auditors.

WESTERN SECTION

The eighth Annual General Meeting of the Section was held at 5.30 p.m. on Thursday, January 10th, 1963, in the University of Bristol. The Chair was taken by the Chairman of the Section, Dr. F. H. Pollard. The following appointments were made for the forthcoming year: *Chairman*—Dr. F. H. Pollard. *Vice-Chairman*—Mr. E. A. Hontoir. *Hon. Secretary and Treasurer*—Dr. T. G. Morris, Brockleigh, Clevedon Avenue, Sully, Glamorgan. *Members of Committee*—Dr. L. E. Coles, Dr. G. V. James, Mr. G. M. Telling, Mr. J. D. R. Thomas and Dr. W. J. Williams. Mr. S. Dixon and Mr. C. H. Manley were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by a Joint Meeting with the Bristol and District Section of the Royal Institute of Chemistry, at which a talk on "New Developments in Chelatometry—A Review of Methods and Reagents Related to EDTA" was given by T. S. West, B.Sc., Ph.D., A.R.I.C. The Chair was once again taken by Dr. Pollard.

Obituary

MARY CORNER

AFTER several months of suffering, Mary Corner died on Sunday, November 4th, 1962, at St. Bartholomew's Hospital. Born on March 25th, 1899, her schooling was delayed for some years by an unfortunate accident, the effect of which was to remain with her for the rest of her life. Her early education was received at Beulah House High School, Balham, which she left in 1915. During the war years she worked in a pharmacy, and then, in 1922, entered Battersea Polytechnic, where she graduated. After spending a further year at London University, she took a post in 1928 at the British Cotton Industry Research Association, thus following Dr. (later Sir) Robert Pickard, whom she greatly admired and who, in 1927, had relinquished the Principalship of the Polytechnic to become Director of the Research Association. Miss Corner first served in the rayon department, and one of the papers published with her colleagues dealt with the microdetermination of metals in commercial rayon yarns (*J. Text. Inst.*, 1933, **24**, 293). This work probably triggered off her interest in microanalysis, and it was not long before she was made head of the microanalytical section. In 1933 she attended the first of two short courses on organic and inorganic micro methods held by Dr. Janet Matthews in the Plant Physiology Department of Imperial College. It was the members of these two courses who formed the Microchemical Club, which flourished until, in 1944, it became the nucleus of the Microchemistry Group of our Society.

Early in 1945 Miss Corner left Manchester to take up a similar appointment with the British Leather Manufacturers Research Association. After a little more than two years, she was invited to become head of the newly formed Microanalytical Section of the Chemical Research Laboratory, later the National Chemical Laboratory. She was pleased to accept the post, which was conveniently sited, for she and her mother were now living in Teddington. An excellent microanalyst herself, she was not only good at instructing those who were assigned to assist her but also could instil into them her own high standard of integrity and her keen interest in microanalysis. More recently Miss Corner had devoted herself mainly to development and research problems. The oxygen-flask method had caught her interest and she quickly used it for the determination of organically-bound arsenic, phosphorus and boron. Sub-micro methods of analysis were also being examined. Always keenly interested in her work, she was ever ready to talk about it and, indeed, to discuss with anybody at any time any aspect of microanalysis. She took a prominent interest in the affairs of the Societies to which she belonged, being Vice-Chairman of the Microchemistry Group at the time of her death, and having served on the Council of the Society for Analytical Chemistry during 1953–54. The work of the British Standards Institution also interested her, and she was Chairman of one of the Sub-Committees dealing with microchemical apparatus. She was made a Fellow of the Royal Institute of Chemistry in 1946, having been elected to the Associateship in 1931.

Miss Corner was an exceptionally fine character and it was a privilege to know her. Burdened with a severe disability, she had had, in addition, more than the usual share of suffering and trouble. Yet she never complained but, on the contrary, she was always cheerful and ever had a thought for the troubles of others. Even to the end she was intensely alive and, in spite of everything, she enjoyed life. In her early years she had even played games and was fond of horse-riding and gardening. A loyal and a generous friend, she has left all those who cherished her friendship much the poorer for her passing.

G. R. DAVIES

Analytical Methods Committee

PARTICLE SIZE ANALYSIS SUB-COMMITTEE

Classification of Methods for Determining Particle Size

A Review*

FOREWORD

THE information presented in this Review was collected by the Particle Size Analysis Sub-Committee† of the Analytical Methods Committee of The Society for Analytical Chemistry during the period March 1961 to December 1962. It forms part of a comprehensive survey of this field of analysis that is being made by the Sub-Committee.

The initial task has been to list and classify existing methods; this classification in tabular form is presented here, together with short explanatory notes on the methods listed. Some form of appraisement or evaluation of methods or apparatus is envisaged for later publication. Meanwhile, it is hoped that this classification may be helpful as a guide to those whose work requires them to practise particle-size analysis, and in particular to those who are studying the subject for the first time.

A. MEASUREMENT OF SIZE DISTRIBUTION

A.I. Relative Motion between Particles and Fluid:

				Method No.
FLUID STATIC (sedimentation)	*Gravitational*	Sedimentation pipettes		1, 2
		Sedimentation columns	Liquid	3, 4, 5, 6, 7, 8
			Gas	9
			Hindered settling	10
		Density variation	Gravimetric	11, 12
			Absorptiometric	13, 14, 15
			Radiometric	16, 17, 18, 19, 20, 21, 22
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			Gas	33, 34, 35, 36,

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ELECTRON BEAM	Electron microscope		52

[*continued*

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* Reprints of this paper will be available shortly. For details, please see p. 247.

† The Sub-Committee consisted of Messrs. E. Q. Laws (Chairman), R. de B. Ashworth, D. G. Beech, C. G. L. Furmidge, H. Heywood, H. W. Hibbott, J. F. Hinsley, R. Howes, R. Jackson and B. H. Kaye, with Miss C. H. Tinker as Secretary.

A. MEASUREMENT OF SIZE DISTRIBUTION. *continued*

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METHODS FOR DETERMINING PARTICLE SIZE

GENERAL INTRODUCTION

POWDERS OF SOLID PARTICLES—

The determination of the size distribution of particles in a powder is not as simple as it might at first sight appear. In addition to any experimental difficulties, there is the basic problem of defining both size and distribution. The variation in the answers obtained for the same powder when different definitions are used can be enormous, and therefore there should be a clear understanding of the definitions to be used before the size analysis is attempted. If powders contained only spherical particles there would be no difficulty in defining particle size, since the size of a sphere is uniquely determined by its diameter. But the particles of a powder are rarely spheres; usually they are irregular in shape and often far from spherical—sometimes being platelets or needle-shaped. Nevertheless, because of the work involved in sizing the large number of particles present in most powders, it is necessary for practical purposes to define the size of each particle by a single parameter. Since no definition can be fully satisfactory, it is usual to select the parameter most relevant to the problem under discussion.

The difficulty in selecting a single parameter can be illustrated by a homely example. A standard brick measures 9 inches × 4½ inches × 3 inches. In comparing the size of this with that of a non-standard brick, say 7 inches × 5 inches × 5 inches, the answer as to which is the bigger depends on the criterion laid down. For example, if the bricks are to be used as an edging to a path, the dimension that matters is the over-all length, then the standard brick is the bigger of the two. If the bricks are to be laid flat to form a pillar, then the larger brick as regards height of the pillar is the non-standard one. The volume or weight of the non-standard brick is also greater than that of the standard one, but the solid diagonal is less. Obviously, the parameter to be used depends on the purposes for which the bricks are intended. The same general arguments apply to all powder particles. If the particles are highly irregular, the problem is even more complex. Parameters often used are, for example, the maximum chord, the projected area, the distance between two parallel tangents and the maximum chord in a given direction. Many other parameters could easily be devised, each having its own value.

For particles with the same shape and density, the free-falling velocity, an important measure in many industrial problems in which solids are mixed with liquids or gases, depends on the size of the particle. In such instances the diameter of the "equivalent sphere" is taken as a measure of size. The equivalent sphere is one with the same density as the particle, which also has the same free-falling velocity as the particle. If the diameter of the equivalent sphere is calculated from the free-falling velocity by using Stokes's law, this is usually referred to as the Stokes's diameter. When the shape or density varies from one particle in a powder to another, the dimensional size is no longer the main controlling factor.

The size distribution can be defined in terms either of the number or the weight of particles within a given size range. It is usually expressed either as the percentages in each of a series of size ranges or as the percentages of the powder under or over a series of specified sizes.

The definitions of size and distribution adopted should therefore be selected first, according to the purpose for which the result is required, for example, whether the weight, covering power, free-falling velocity or some other parameter, is the most important in the problem and second, allied to this, on the particular form of instrument to be used to measure the size distribution. Most methods supply only one parameter. If the method is not well chosen the results may not be directly comparable with the particular property being investigated. In the foregoing tables—"Classification of Methods"—the size-determination technique is arranged first according to the parameter investigated and secondly according to the method.

Finally, it should be appreciated that both the collection of a sample and also the act of measurement (for example, if this involves the use of ionising radiation such as X-rays) may alter the condition of the material. Fine particles tend to agglomerate into larger clusters that may not always be easy to distinguish as such or to re-disperse, before measurement, into the original individuals. Coarse particles are sometimes friable and break into smaller particles during collection or measurement. In all cases, and particularly when a powder is produced or used in the dispersed condition, considerable care should be given in interpreting any of the results obtained by any measuring system.

LIQUID DROPLETS—

If the particles to be investigated are not solid but are in the form of liquid droplets, many methods do not apply and others may have to be modified. Sample collection is much more difficult and any method that allows the particles to touch and so coalesce is inadmissible. When collected on microscope slides or on an impervious surface, an extremely low density of droplets is necessary, since superposition must be avoided. The droplets will be distorted by contact with the surface, but correction for this may be made by determining the spread factor; since this will vary with droplet size it must be determined in the relevant size range. Collection on thin filter-paper may also be used and correction factors similarly determined. A more accurate method is to use relatively thick filter-paper or magnesium oxide or plaster of Paris blocks. In other techniques, droplets have been frozen in a stream of cold air before collection, and then treated as solid. Another ingenious way of solidifying drops is to allow them to fall into talc, wheat flour or other finely powdered material, and to determine the size of the lumps of agglomerated "dough" produced. Another method is to allow the drops to impinge on glass slides coated with magnesium oxide smoke from which the drops bounce, taking with them a piece of magnesium oxide and leaving a crater proportional to the size of the drop. This method is useful in the field, since the slides may be examined later at leisure.

Some workers have caught water drops in a cell in which a density gradient has been produced by forming a carbon tetrachloride layer beneath light petroleum. Care must be taken to avoid too great a gradient so as to avoid distortion of the droplets; only a few droplets may be collected at a given time, since they may coalesce if they are too close.

A combination of settling and collection on a moving belt of filter-paper has been used for accurate sizing, and the Conifuge (see (**42**)) has been used for similar work.

SELECTION OF METHODS—

Most of the methods given in this Classification cater for particles in the sub-sieve size range. Systems in which the individuals are resolvable by the naked eye are normally classified by sifting; the practical lower limits of woven-wire sieves is usually taken as 200 mesh—this corresponds to a nominal particle size of 76 μ, but, in exceptional circumstances, this lower limit can be extended down to 300 mesh (53 μ).

Before beginning a size determination it is essential to *look* at the powder. Often the naked eye or a low-power magnifying glass may be sufficient, but in general it is preferable to examine the material under a microscope. This examination should reveal the approximate size range and distribution of the powder, its uniformity of composition, the shapes of the particles and the presence of aggregates. All these factors may influence the further steps to be taken. Of particular importance is the presence or otherwise of aggregates; it is necessary to know if they are present and in the same manner in the sample as in the original form. If information on this point is not available, this fact should be reported.

In most methods of sizing, the powder has to be prepared in a suitable way. The presence of aggregates may mean that these have to be broken down into their constituent parts or, on the other hand, that they must not be broken down because they are an essential feature of the material. Usually, the individual particles or aggregates have to be separated from their fellows in order to permit individual sizing. This is necessary when a microscope is being used, so as to avoid overlapping or obscuration of particles. In sedimentation, it is necessary to ensure that each particle behaves as if it were alone, since increasing concentration causes the mass to behave as a whole, not as individual particles. For each of the sizing techniques there is an appropriate method of preparation and a range of concentrations; these must be carefully observed.

When deciding which method of sizing shall be used for a given problem there are both theoretical and practical considerations to be taken into account. From what has been said earlier, it is clear that the first and most important consideration must be the purpose for which the information is required. Knowledge of free-falling speeds, as in estimating the behaviour of powders in classifying or separating machines, can best be obtained from a sedimentation or elutriation technique; covering power of a powder is related to the projected area size as seen, for example, under the microscope; other types of required behaviour are represented most nearly by the appropriate size, determined, possibly, by other techniques.

When the most desirable type of technique has been selected, the choice can be narrowed by finding the instrument in this class that gives the necessary accuracy, covers the range of sizes of interest and is appropriate to the problem. Sedimentation, for example, requires a test fluid, and it may be difficult to find one that is both suitable for the apparatus and does not affect the powder. To take another example, light or X-rays can be used to size particles but are inappropriate for, say, silver bromide grains, since during the course of the test they would alter the nature of the material.

Lastly, but by no means least, the availability of appropriate equipment is important. This may be the controlling factor in selection of a method, since only a single instrument may be available. It must then be fully appreciated that the "size" determined by this instrument is not necessarily the true size. In some instances the result may be correct; in others, a simple correction factor, say $\sqrt{2}$, may produce a close approximation to the correct answer; in a number of instances, however, the determined "size" may bear only a vague relation to the one needed, and may be completely misleading. Such methods, if reproducible, may nevertheless be of value for process control purposes.

A.I. RELATIVE MOTION BETWEEN PARTICLES AND FLUID

SEDIMENTATION PROCESSES

Sedimentation analysis is a process in which the rate of settlement of particles in a static fluid is measured, the analysis being either with the particles uniformly dispersed throughout the whole of the fluid, or introduced at the top of the column, as in (**5**) and (**10**). The rate of sedimentation usually follows Stokes's law, and settlement may be under conditions of gravitational acceleration or under centrifugal acceleration, imposed by rotation, in order to increase the rate of sedimentation. Stokes's law is valid only in the regime of viscous flow, which sets an upper limit to the size of particle that can be tested by this means in a given fluid. The limit is determined by the magnitude of Reynolds number, which should not exceed 0·2 if the error when Stokes's law is used is not to exceed 5 per cent. The concentration of the suspension should always be as low as possible, except for method (**10**), in order to avoid interference between particles, particularly since "clouds" of particles tend to move *en masse* and not individually. The method of measurement, *e.g.*, by weighing, may dictate a lower limit of concentration beyond which inaccuracies become unacceptably high.

Effective dispersion of the particles is an essential pre-requisite for all methods of sedimentation analysis. It is often necessary not only to stir the suspension vigorously, but to introduce the powder already mixed with a dispersing agent. A further agent to prevent flocculation may be needed, and it may be necessary to adjust the pH of the suspension to a suitable value determined by experiment.

A suspension uniformly dispersed in an upright cylindrical vessel at time $t = 0$ begins to settle immediately. If two horizontal planes, p_1 and p_2, are taken at depths h_1 and h_2 below the upper surface ($h_1 > h_2$), then, at time t, the mass of suspension remaining between p_1 and p_2 will be determined by—

$(h_1 - h_2)\,\Sigma$ *plus* particles that have fallen through p_2 *minus* particles that have fallen through p_1,

where Σ = mass per unit depth of original suspension.

For incremental methods of analysis, the sample is theoretically taken from an extremely thin layer in which h_2 is nearly equal to h_1; for cumulative methods of analysis, the amount settling in a particular plane in a given time, normally at the bottom of the settling vessel, is measured.

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Stairmand, C. J., "Symposium on Particle Size Analysis." Supplement to *Trans. I. Chem. E.*, 1947, **25**, 128.
Donoghue, J. K., *Brit. J. Appl. Phys.*, 1956, **7**, 333.
British Standard 3406 : Part 2 : 1962.

Pipette Methods

In this technique a sample is extracted from the sedimenting suspension at appropriate intervals by means of a pipette. With the exception of the Whitby Column Method (**5**), these methods are all incremental. The sample is taken in one of two ways: (*a*) at a fixed position in the apparatus or (*b*) at a fixed depth below the surface of the suspension. It is assumed in both instances that no disturbance of the suspension takes place by eddies, etc., while the sample is being taken, that the sample is representative of the suspension at the extraction point and that the sample taken is small. Method (*a*) must take into account any lowering of the level of the top surface of the suspension.

(**1**) ANDREASEN PIPETTE—

This is probably the most frequently used method of sedimentation analysis, the disadvantage being that samples withdrawn must be evaporated to dryness and weighed —a time-consuming procedure.

The glass sedimentation vessel is about 5·5 cm in diameter and has a graduated scale, 0 to 20 cm, engraved on its side. The zero of the scale is positioned about 2·5 cm from the base of the vessel, and the capacity when filled to the 20-cm mark is 550 to 600 ml. The stem of the pipette is fused to a bell-shaped dome having a ground-glass joint that fits the neck of the sedimentation vessel, the pipette being so positioned that its tip is fixed at the level of the zero mark of the scale; above the dome is a two-way tap and a side discharge tube. During an analysis, the sedimentation vessel is immersed in a constant-temperature bath up to the 20-cm mark.

The vessel is filled with suspending liquid and powder to the 20-cm mark, shaken, and placed upright in the bath. At time intervals standing in a 2 to 1 progression, a 10-ml sample is withdrawn from the sedimentation vessel and is discharged into a series of tared dishes. The dishes are reweighed after the samples have been evaporated to dryness, and a deduction is made for the weight of dispersing agent added; thus, the weight of particles corresponding to each withdrawal time is determined.

The size of particles is calculated from the height of fall and time elapsed, according to Stokes's law, but allowance must be made for the decrease in the height of sedimentation column after each sample is withdrawn. The initial concentration in the suspension is calculated from the weight of powder and the volume of liquid in which it has been dispersed. This concentration may be up to 1 per cent. by volume for easily dispersed powders, but should be reduced if there is any tendency for flocculation to occur.

SIZE RANGE—

The apparatus is normally applicable to the particle size range 2 to 60 μ, and may be extended to larger sizes if viscous liquids are used, *e.g.*, benzyl alcohol, or to smaller sizes if a constant temperature is maintained over the longer period required. One operator can perform four analyses simultaneously during a day. The weight of powder required is approximately 5 g.

REFERENCES—

Andreasen, A. H. M., *Kolloid-Beih.*, 1928, **27**, 349.
——, *Ingen Vidensk. Skr.*, 1939, No. 3.
Heywood, H., *Proc. Soc. Mech. Eng.*, 1958, **140**, 257.
British Standard 3406 : Part 2 : 1962. Fixed position pipette method.

(**2**) FIXED-DEPTH PIPETTE (B.S. 1377)—

The apparatus consists of a 10-ml pipette fitted with a two-way tap and a washing bulb. The pipette is mounted on a sliding panel so that it can be lowered to any depth in the sedimentation vessel.

The general method of procedure is similar to that of (**1**), except that the pipette is removed from the suspension after each sample has been withdrawn and is re-immersed to a fixed depth below the surface of the suspension liquid for the next sample. An advantage over the Andreasen pipette is the facility for washing the pipette with clear liquid to remove any deposited particles.

SIZE RANGE—

This is similar to (**1**)—2 to 60 μ. The operating time is also similar, and the weight of powder required is approximately 5 g.

REFERENCES—

British Standard 1377 : 1948.
British Standard 3406 : Part 2 : 1962. Fixed depth pipette method.

Sedimentation Columns: (a) Liquid

In these methods the powder may be initially dispersed in the sedimentation liquid or added, effectively instantaneously, at the upper free surface. The rate of fall of the powder is measured as the rate at which powder reaches the bottom of the column. It may be physically removed from the column, if this has an openable base, or measured as the separating sediment in a column with a closed bottom.

(**3**) LIQUID COLUMN METHODS WITH SEDIMENT EXTRACTION—

The sedimentation vessel is of cylindrical form with a conical base leading to $\frac{5}{16}$-inch bore glass tube; just below the cone a branch tube, $\frac{5}{16}$-inch bore, joins the vertical tube at right angles and is connected to a liquid reservoir. The sedimentation vessel is filled with the liquid and dispersed powder, which is stirred until the beginning of the test by compressed air admitted through the outlet tube at the bottom.

Immediately after the start of sedimentation, a centrifuge tube is connected to the outlet tube from the sedimentation vessel by means of a short length of rubber tubing fitted with a spring clip. Settled particles collect above this clip, and, at suitable time intervals, usually about 1, 2, 4, etc., minutes from the beginning of the analysis, these particles are run off into the centrifuge tube, which is replaced by another tube in readiness for the next extraction. The liquid withdrawn is replaced from the clear-liquid reservoir, so that the height of the suspension column remains constant. The particles collected in the tubes are compacted in a centrifuge, washed if necessary, dried, and weighed to determine the weight of particles collected.

The size of particles corresponding to each time interval is calculated by means of Stokes's law, and the corresponding weights are determined by a tabular method of calculation, which allows for the fact that total sediment has been collected. It is essential for the validity of this calculation that the time interval be exactly in a geometrical progression of ratio 2 to 1.

SIZE RANGE—

The apparatus is applicable to the size range 3 to 76 μ, and the particle concentration may be of the order of 0·1 per cent. by volume. The weight of powder required is 0·5 g or less.

REFERENCES—

Stairmand, C. J., "Symposium on Particle Size Analysis." Supplement to *Trans. I. Chem. E.*, 1947, **25**, 128.
British Standard 3406 : Part 2 : 1962. Liquid column method with sediment extraction.

(4) B.C.U.R.A. SINGLE TUBE—

The form of the tube is similar to that described under (**3**), with some detailed alterations. The conical lower portion contains no side tube, and the tip of the cone is ground away to leave a hole about 1 mm in diameter. When the tube is filled with liquid and the upper end closed by the tap, the liquid is held in the tube by the meniscus across the small hole at the bottom. Any sediment collects at this hole. When clear sedimenting liquid, contained in a small glass bucket, is brought into contact with the meniscus, this breaks down and sediment passes into the bucket. Removal of the bucket, after the appropriate time interval, takes away the sediment, but the sealing meniscus again forms. The process is repeated for the number of samples required.

Initial dispersion of the powder by compressed air blown through the liquid, the treatment of the sediment samples and the final calculations are essentially similar to those for (**3**).

SIZE RANGE—

This is similar to (**3**)—3 to 76 μ.

REFERENCE—

Kobak, J., and Loveridge, D. J., *J. Sci. Instrum.*, 1960, **37**, 266.

(5) WHITBY COLUMN METHOD

The main features of this method are that it uses gravitational settlement followed by centrifugal sedimentation, and the sample suspension is superimposed on a column of clear liquid so that all the particles begin to settle from the same level, provided that the sample layer is thin. The suspending medium is chosen to have a density slightly less than that of the sedimentation liquid. The amount of solid sample required for the analysis is a few milligrams.

The apparatus consists of a glass centrifuge tube, 14 mm internal diameter, ending at the bottom in a graduated capillary tube, 1 mm internal diameter. The length of the upper portion is about 65 mm, and the capillary, which is 30 mm long, is sealed to the upper 14-mm diameter tube by a smooth glass connection. The over-all length of the apparatus is approximately 125 mm, including the junction. A feed-tube is provided for the introduction of the sample. This is a short metal tube that fits easily into the mouth of the glass centrifuge tube. A 40-mesh wire gauze is soldered across the bottom of the metal tube. This feed-tube is fitted with a collar that restricts its entry into the centrifuge tube to a depth of 10 mm.

In operation, the centrifuge tube is filled with the sedimentation liquid to within 10 mm of the top. The feed-tube is then filled with the sample suspension and the upper end is closed by the operator's finger. The feed-tube is then placed in the centrifuge tube with the suspension in contact with the column of clear sedimentation liquid; this starts the sedimentation. The finger is then removed.

Gravitational settlement is allowed to proceed for 4 to 10 minutes, according to the particle-size range of the sample. The tube is then transferred to a suitable centrifuge, and a series of readings is taken at known intervals at several measured centrifuge speeds between 300 and 4000 r.p.m. The amount of material that has settled in the capillary tube is measured after each experiment by reading the volume of the sediment in the capillary tube. The relative volume is a direct measure of the amount of material having a particle size greater than that calculated from the time of settlement and the gravitational force applied.

A feature of the method is that, since the suspension medium is less dense than the sedimentation liquid, an unstable density gradient is avoided, and the particles begin to settle according to their relative terminal velocities.

SIZE RANGE—

0·05 to 100 μ.

REFERENCE—

Whitby, K. T., "A Rapid General-purpose Centrifuge Sedimentation Method for Measurement of Size Distribution of Small Particles," *A.S.H.A.E. J. Heating, Piping & Air Conditioning*, 1955.

Sedimentation Balances

These instruments, sometimes referred to also as sediment accumulation devices, weigh the sediment as it accumulates on a weigh-pan at the base of the sedimentation column. The methods are cumulative ones. The powder may be dispersed initially in the bulk of the fluid or added instantaneously at the top. An advantage of this type of equipment is the absence of the conical base, needed in sediment extraction devices, upon the walls of which some sediment may stick. The danger of particles sticking to the vertical walls is however still present.

(6) BOSTOCK SEDIMENTATION BALANCE—

The sedimentation vessel is cylindrical, water-jacketed and surmounted by a cylindro-conical pre-mixing and feed vessel. Below the open end of the sedimentation vessel is a balance pan supported by a torsion wire with an extended pointer, scale and optical magnifying system, such that 0·5 g of the material on the balance pan will cause full-scale deflection of the system. The liquid and dispersed powder are introduced into the sedimentation vessel via the feed vessel, and readings of the torsion balance (which represent the weights of particles settled on the pan) are taken at specified time intervals. The weights of particles corresponding to the various particle sizes, calculated from the time intervals, are determined by the tabular method as for (**3**) or by a graphical method given by references below.

SIZE RANGE—

3 to 76 μ. The weight of powder required is 0·5 g.

REFERENCES—

Bostock, W., *J. Sci. Instrum.*, 1952, **29**, 209.
Donoghue, J. K., *Brit. J. Appl. Phys.*, 1956, **7**, 333.
British Standard 3406 : Part 2 : 1962.

(7), (8) SARTORIUS AND SHIMADZU SEDIMENTATION BALANCES—

These instruments are automatic balances of the beam type. The balance pan is hung in a suspension of appropriate concentration, and the balances record the change in weight as powder settles on the pan. When the deflection of the beam reaches a certain point, corresponding to a given weight increment, the beam is brought back to equilibrium and the recording pen moves an appropriate distance on the weight axis of the chart. As counterpoise, the Sartorius balance has an auxiliary torsion beam and the Shimadzu balance has small steel balls. Provision is made in both instruments for varying the time scale.

The instruments produce a step-wise record; a curve is then drawn through the tops of the steps and the cumulative size distribution is determined by the Oden tangent method. In order to apply this method, it is necessary to know the amount of material settling at infinite time (*i.e.*, the total amount of material in suspension). With the Sartorius balance this may be determined directly, provided the material is not too fine, or may be calculated from the weight of the sample by applying an empirical correction for the material that escapes the balance pen. With the Shimadzu balance, it is recommended that the "unsedimented powder" should be estimated from a sample of suspension siphoned off at the end of the experiment and added to the final weight of sediment to give the amount at infinite time.

SIZE RANGE—

1 to 60 μ.

REFERENCES—

Oden, S., *Intern. Mitt. Bodenk.*, 1915, **5**, 257.
Oden, S., *in* Jerome Alexander, *Editor*, "Colloid Chemistry," Chemical Catalogue Co., New York, 1926, p. 861.
Rose, H. E., and Langmaid, R. N., *Nature*, 1957, **179**, 774.

Sedimentation Columns: (b) Gas

(9) MICROMEROGRAPH—

This commercial instrument is operated on the principle of sedimentation of particles in a static column of air. The powder is dispersed and injected at the top of the column by compressed nitrogen, so that all particles are assumed to begin settling from the same level. The settled particles are collected on a balance pan at the base of the air column.

The weight of particles is recorded by an automatic servo-controlled, electrically-operated torsion balance, and a curve of weight of settled particles against time is obtained; this can easily be converted to relate weight and particle size. The sample weight required is 50 to 100 mg.

SIZE RANGE—

$0{\cdot}8$ to 250 μ.

REFERENCE—

Eadie, F. S., and Payne, R. E. *Brit. Chem. Eng.*, October, 1956.

Sedimentation Columns: (c) Hindered Settling

(10) HINDERED SETTLING METHOD—

In suspensions with a concentration of more than about 1 per cent. by volume, the particle fall becomes progressively hindered until, at a concentration of about 18 per cent., the whole suspension settles *en masse*. A clear boundary forms between the settling suspension and the fluid draining out of the slurry. The rate of settlement of the boundary can be related to surface area of the powder by considering the situation to be the flow of fluid through a packed bed rather than the settling of particles.

The mean specific surface diameter can be calculated from the formula—

$$d_s = 12{\cdot}4 \times \left[\frac{\mu u_0 (1 - \epsilon)}{g(\rho - \rho_0)\epsilon^3}\right]^{\frac{1}{2}}$$

where d_s = mean specific surface diameter;
ϵ = porosity of suspension;
ρ = density of particles;
ρ_0 = density of fluid;
g = acceleration due to gravity;
μ = viscosity of fluid;
u_0 = velocity of boundary.

The method is easy to apply, since only two measurements are required—the porosity of the suspension and the velocity of the boundary. It also has the advantage that a large sample of powder is required so that sampling error is minimised.

SIZE RANGE—

5 to 1000 μ.

REFERENCE—

Orr, C., jun., and DallaValle, J. M., "Fine Particle Measurement," The Macmillan Co., New York & London, 1959.

Density Variation: (a) Gravimetric

(11) HYDROMETER METHOD—

The suspension of a powder is prepared as for other sedimentation analyses and poured into a 1-litre glass measuring cylinder. The variation with time of effective density, and hence of particle concentration, is determined from readings of a hydrometer immersed in the suspension. The hydrometer should be inserted in the suspension immediately before a reading is taken, and afterwards removed to avoid particle deposit on the bulb. The effective height of the suspension is from the surface level to the centre of volume of the bulb, which varies according to the density of the suspension. As the bulb length is an appreciable proportion of the sedimentation height, and not very small, as explained in the Introduction to section A.I (see p. 161), the incremental method of calculating the size analysis is approximate only. Temperature and meniscus corrections should be applied. The method is frequently used for soils, and full descriptions can be found in any recognised text-book on this subject.

SIZE RANGE—

1 to 25 μ.

REFERENCE—

British Standard 1377 : 1948. Methods of test for soil classification and compaction.

(12) DIVER METHOD—

This is a modification by Berg of the hydrometer method (**11**). Variation in effective density, and hence concentration, is measured by totally immersed divers. These are small glass vessels of approximately stream-line shape, ballasted to be in stable equilibrium, with the axis vertical, and to have a known density slightly greater than that of the sedimentation liquid. As the particles settle, the diver moves downwards in hydro-dynamic equilibrium at the appropriate density level. The diver indicates the position of a weight concentration equal to the density difference between the diver and the sedimentation liquid. Several divers of various densities are required, since each gives only one point on the size-distribution curve.

The advantages claimed for this method over the hydrometer method are that, since the divers are relatively small and surface tension and deposition effects are avoided, the incremental theory is applicable. The method is simple to operate, and accurate when a set of divers has been prepared. Smaller divers can be used with centrifuge tubes, so that analysis can be extended down to particles of 0·02 μ in size. A modification by Jarrett and Heywood permits the position of the diver in an opaque suspension to be determined electronically.

Particles deposited on the upper surface of a spherical diver produce a state of unstable equilibrium such that a slight displacement causes the diver to rotate about a horizontal axis and shed some of the particles. This is an advantage in that the weight of the diver remains nearly constant.

SIZE RANGE—

0·02 to 40 μ.

REFERENCES—

Berg, S., *IngenVidensk. Skr.*, 1940, No. 2; *A.S.T.M. Special Technical Publication No.* **234**, 1958, p. 143.

Jarrett, B. A., and Heywood, H., *Brit. J. Appl. Phys.*, Supplement No. 3, 1954, S21.

Density Variation: (b) Absorptiometric

The presence of a suspension of particles in a cell through which a beam of light or other radiation is passed causes a reduction in intensity of the emergent radiation. This principle is used to follow the changes in particle concentration at various depths below the surface in a sedimentation cell.

(13) "E.E.L." PHOTOSEDIMENTOMETER—

Light from a 36-watt lamp is projected through a system of lenses and stops, so that a beam of extremely small solid angle (0·00022 radian) is subtended in a position occupied by a sedimentation cell. A barrier-layer photo-cell receives the transmitted light; the output of this cell is passed to a galvanometer. The optical components are mounted on a movable carriage that can be traversed so that any one of six cells may be examined, the cells remaining stationary. One cell contains clear suspending medium, and the rest may contain five different samples, each dispersed in the medium. Readings of the galvanometer, in respect of each cell, are taken at fixed time intervals.

SIZE RANGE—

1 to 50 μ.

(14) "BOUND BROOK" PHOTOSEDIMENTOMETER—

This instrument employs a single light source sending a beam to two matched photo-cells. The sedimentation cell is placed in one of the optical paths and a reference cell of clear liquid in the other. The two photo-cell signals are passed to an automatic pen recorder consisting essentially of a slide wire and a resistor in series, the two signals being continually balanced and the ratio of the signals recorded, thereby preventing errors consequent on light source variation.

SIZE RANGE—

1 to 50 μ.

Radiometric Techniques

The specialised methods of measurement developed for use with radioactive tracers and other active materials are highly sensitive. They may be applied to particle-size analysis and are well adapted for use with sedimentation processes.

(*i*) *Penetrating radiation*—Penetrating radiation has the merit of being effective when dispersing media opaque to visible light are being used. The types of radiation referred to below are of much shorter wavelength than visible light and have high energy and great penetrating power—extending to several inches of steel.

(*ii*) *Electromagnetic radiation*—X-rays and gamma-rays form part of the spectrum of electromagnetic radiation and are propagated with the velocity of light. The usual source of X-rays is a tube in which a target is bombarded by high-velocity electrons. The type of radiation is continuous, with superimposed high-intensity lines characteristic of the target element. It has a short wavelength limit related to the maximum electron velocity. Gamma-rays have a line-type spectrum; they are conveniently available from radioactive isotopes and have a constant quality, although the intensity diminishes with time.

(*iii*) *Atomic particles*—Alpha-rays are high-speed helium particles travelling with up to one-sixteenth of the velocity of light; beta-rays are electrons travelling with velocities approaching nine-tenths of that of light. Both these types of radiation are available from radioactive materials: alpha-particles carry a positive charge, electrons a negative charge. Neutrons, which are available from reactors, particle accelerators and special compact sources, carry no charge; "thermal" neutrons are most likely to be useful in particle-size determination, and have an equivalent wavelength of a few Ångstroms.

The material under examination may be initially radioactive or may have radioactivity induced in it by irradiation or by incorporation of tracers. Alternatively, radiations may be used as an external analytical probe. The techniques offer several advantages; some of these are—

(1) Very high sensitivity permits the use of extremely low particle concentrations.

(2) A settling system can be studied remotely without introducing disturbances associated with sampling devices.

(3) Radiometric techniques are convenient for use in conjunction with a centrifuge for sizing particles down to 0·1 μ—a range in which optical methods are not practical.

(4) Sedimentation can be followed in opaque systems, such as liquid metal slurries.

(5) The sedimentation rates of components of mixtures can, in favourable instances, be followed concurrently.

(15) I.C.I. METHOD, INVOLVING USE OF X-RAYS—

This method is suitable for material that is chemically homogeneous. An X-ray beam is split into two separate beams, each of which ultimately falls on to a device for converting X-rays into longer wavelength radiation, such as visible light. Fluorescent screens or crystal scintillators are suitable; the longer wavelength radiation is conveniently monitored for intensity by a photo-electric device. The use of X-rays has the advantage over light methods in that absorption is directly related to the mass of a larger variety of absorbing material, with consequent increase in sensitivity.

It is thus possible, by the X-ray method, to complete a particle-size analysis of material such as fly-ash (size range 7 to 76 μ) in about 30 minutes, whereas the normal sedimentation method takes 3 to 4 hours. The method also has the advantage of a direct continuous measure of the concentration of the settling powder with no disturbance of the suspension.

Besides fly-ash, substances that have been examined include iron oxide and bismuth oxide, in concentrations between 0·2 and 0·3 per cent. by weight (or 0·15 and 0·04 per cent. by volume).

SIZE RANGE—

1 to 76 μ.

REFERENCE—

Brown, J. F., and Skrebowski, J. K., discussions associated with Jarrett and Heywood; "The Physics of Particle-size Analysis" (Institute of Physics, Nottingham, 1954, Supplement No. 3, S21).

(16) INCREMENTAL SEDIMENTATION (RADIOMETRIC)—

The technique consists in measuring the changing gamma-activity of a cross-sectional lamina of a sedimenting gamma-active suspension. The gamma-activity is directly proporportional to the mass of active material in the lamina, which is defined by a collimating slit in a lead shield surrounding the sedimentation tube and a scintillation counter.

A cumulative distribution curve of the mass-fraction of undersize particles is derived from the measurement of gamma-activity at appropriate time intervals.

SIZE RANGE—

4 to 76 μ.

REFERENCES—

Abraham, B. M., Flotow, H. E., and Carlson, R. D., *Anal. Chem.*, 1957, **29**, 1058.

Connor, P., and Hardwick, W. H., "The Use of Radioactivity in Particle-size Determination," *Indust. Chemist*, 1960, September.

(17) BEAKER-CENTRIFUGE (RADIOMETRIC)—

A tracer technique is used in conjunction with Martin's centrifugal method. The activity of the tracer is induced by neutron irradiation. Portions of the irradiated suspension are placed in calibrated centrifuge tubes, and each sample is spun in a centrifuge for a predetermined time. Portions of the supernatant liquid are removed and counted with a gamma-scintillation counter so that the percentage concentration of undersize particles in the suspension may be calculated. The method is stated to be sensitive to particles down to 0·05 μ in size.

The sedimentation rates of the constituents of a mixture of several particulate materials can be determined if neutron irradiation of the constituents produces a set of gamma-emitters that can be differentiated by gamma-spectroscopy, for example, by using a kick-sorter. A mixture of substances containing chromium, iron, zirconium and uranium has been size analysed by this method.

SIZE RANGE—

0·05 to 76 μ.

REFERENCES—

Martin, S. W., *Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 47.

Bate, L. C., and Leddicote, G. W., Oak Ridge National Laboratory Microcard 57–1–116.

(18) TRACERS IN ANDREASEN AND LONG-ARM CENTRIFUGAL PIPETTES—

Low, and therefore free-settling, concentrations of thoria traced with protactinium-233 have been size analysed with an Andreasen pipette (1) and also Gupta's long-arm centrifugal pipette. A fast and accurate procedure was evolved in which portions of the sample were transferred by pipette directly into flat-bottomed plastic centrifuge tubes, which were then spun in a centrifuge to ensure deposition of the suspended solid on the tube base. The activity of the sediment was determined by means of a gamma-scintillation counter. The measurements take the place of the sediment weights usually derived by evaporation to dryness of withdrawn samples.

SIZE RANGE—

0·1 to 76 μ.

REFERENCES—

Andreasen, A. H. M., *Kolloid-Beih.*, 1928, **27**, 349.
——, *Ingen Vidensk. Skr.*, 1939, No. 3.
Gupta, A. K., *J. Appl. Chem.*, 1959, **9**, 487.
Connor, P., Hardwick, W. H., and Laundy, B. J., *Indust. Chemist*, 1960, September.

(19) TWO-LAYER RADIOMETRIC SEDIMENTOMETER—

This is an adaptation of the Whitby Column Method (**5**). It was developed by Connor, Hardwick and Laundy for determining the size distributions of thoria and other materials in the range 0·1 to 5 μ. A few milligrams of thoria powder are dispersed in a dilute solution of trisodium polyphosphate in a small polythene bottle. The bottle, with contents, is irradiated in a flux of $1{\cdot}47 \times 10^{12}$ neutrons per sq. cm per second for a few seconds, so that virtually the only effect is to produce beta-active thorium-233 with beta-energy of 1·23 MeV.

After further agitation of the suspension, a portion containing about 2 mg of thoria is transferred, as in the Whitby Column method, by a special pipette on to the surface of a 9-cm column of concentrated sucrose solution contained in a tube machined from solid Perspex. The flat base of this tube is made thin (0·015 inch) to permit beta-radiation to pass through for recording by a beta-scintillation counter. The activity, and hence the relative mass of the sediment, is measured after various periods of centrifugation. A size distribution is easily determined from the results, because all the particles start from the same height. The method has been successfully applied to silica, lithopone and poly(vinyl chloride), the active isotopes being silicon-31, barium-139 and chlorine-38, respectively.

SIZE RANGE—

0·1 to 5 μ.

REFERENCES—

Marshall, C. E., *Proc. Roy. Soc. A*., 1930, **126**, 427.
Whitby, K. T., *A.S.H.A.E. J. Heating, Piping & Air Conditioning*, 1955, **27**, 139.
Connor, P., Hardwick, W. H., and Laundy, B. J., *J. Appl. Chem.*, 1958, **8**, 716.

(20) BETA BACK-SCATTERING SEDIMENTOMETER—

In this sedimentometer developed by Connor, Hardwick and Laundy, an external source of beta-radiation is used. The mass of sediment on the bottom of a tube is determined by measuring the amount of radiation that it scatters back from a 1-millicurie strontium-90 radioactive source. A special source holder and sedimentation tube are used in a fitting designed to maintain a consistent geometrical arrangement of these parts.

For fine particles, less than 5 μ in size, a centrifuge tube may be used to accelerate settling—a short settling height or a long-arm centrifuge being used. Since the penetrating power of beta-rays is relatively low, the shielding requirements are modest.

A curve of cumulative weight deposited against the logarithm of the settling time is obtained. The size distribution is calculated by application of Gaudin's modification of an expression derived by Oden—

$$W = P - \frac{\mathrm{d}P}{\mathrm{d}(\log t)}$$

where P is the fraction deposited in time t and W is the fraction of material greater in size than the Stokes's diameter corresponding to t.

The value of $\frac{\mathrm{d}P}{\mathrm{d}(\log t)}$ is obtained by differentiating the curve. The method of drawing tangents (see (**7**) and (**8**)), the accuracy of which has been discussed by Donoghue, or numerical methods may be used.

SIZE RANGE—

0·1 to 76 μ.

REFERENCES—

Connor, P., Hardwick, W. H., and Laundy, B. J., *J. Appl. Chem.*, 1959, **9**, 525.
Oden, S., *Proc. Roy. Soc. Edin.*, 1915, **36**, 219.
Donoghue, J. K., *Brit. J. Appl. Phys.*, 1956, **7**, 333.
Gaudin, A. M., Schuhmann, R., and Schlechter, A. W., *J. Phys. Chem.*, 1942, **46**, 903.

(**21**) GAMMA-RAY ABSORPTION—

A sedimentometer making use of X-ray absorption has been described in (**15**). By using a stable gamma-ray source, an instrument independent of supply-mains fluctuations can be constructed. Ross has developed a method in which americium-241 is used as a source of gamma-rays. The strength of the source was 150 millicuries and the energy of the radiations corresponds to 60 keV, with a half-life of 470 years.

SIZE RANGE—

0·1 to 76 μ.

REFERENCE—

Ross, C. F., *Anal. Chem.*, 1958, **31**, 337.

(**22**) BETA-RAY ABSORPTION—

Connor and Hardwick have proposed a method of measuring the sedimentation rate of fine powders from a gaseous suspension. Their beta-sedimentometer incorporates a Perspex centrifuge tube in the extended base of which is placed a disc of organic phosphor. Above the disc is an aluminium shielding ring surmounted by a ring-shaped beta-ray source, both of these being let into the tube wall. A deposit on the base of the tube absorbs some of the beta-radiation that would otherwise reach the phosphor. By using a photomultiplier in conjunction with the phosphor, such decrease of activity can be recorded and related to the mass of the deposit.

SIZE RANGE—

0·1 to 5 μ.

REFERENCE—

Connor, P., and Hardwick, W. H., *Indust. Chemist*, 1960, September.

Sedimentation Rate

(**23**) PHOTOGRAPHY OF PARTICLE TRACK—

The particles in extremely dilute concentration are allowed to settle in a glass cell and are powerfully illuminated by a light beam at right angles to the axis of an optical photographic system. The illumination is limited to a definite plane perpendicular to the optical axis, and is restricted to a specific exposure time. The individual particles are photographed as tracks whose length is proportional to the sedimentation velocity. These tracks are measured on the photographic plate and the number - size relationship is determined for the particles.

SIZE RANGE—

0·5 to 300 μ.

REFERENCE—

Carey, W. F., and Stairmand, C. J., *Trans. Inst. Chem. Eng.*, 1938, **16**.

Fractional Decantation Methods

(**24**) BEAKER METHOD—

The simple beaker method of separating a powdered material into fractions limited by selected particle sizes can be effective if skilfully performed. An appropriate amount of powder is dispersed in liquid in a tall beaker or large measuring cylinder and allowed to settle for a time corresponding to the smallest separating size selected. At the end of this time, the liquid is decanted without disturbing the collected sediment. Ideally, all particles removed in this liquid would be smaller than the selected size. Since many of these smaller particles remain in the sediment, an infinite number of decantations is required to effect complete separation. It has been shown by Heywood that a 90 per cent. separation of particles smaller than the selected size is given by 11 decantations.

When the first stage of separation is regarded as sufficient, the process is repeated with a longer settling time according to the next selected size, and so on. The particles are collected and weighed.

This method of separation cannot be regarded as an accurate sizing process, but it produces fairly large amounts of approximately graded material. The procedure is improved if the liquid is decanted through a siphon tube, or as described for the apparatus in (**25**), (**26**) and (**27**).

SIZE RANGE—

10 μ upwards.

REFERENCE—

Heywood, H., "Recent Developments in Mineral Dressing," *Inst. Min. Met.*, 1952, 31.

(**25**) C.P.A.C. METHOD—

In the examination for suspensibility of water-dispersible powders, the method specifies that a suspension of known concentration is prepared under prescribed conditions with standard hard water. If the manufacturer of the pesticide product recommends creaming when the material is prepared for use, then a sample in a beaker is mixed into a smooth paste with hard water before dilution to 250 ml to produce the suspension; when creaming is not mentioned in the manufacturer's instructions, a sample is added to hard water in a beaker and stirred to produce a similar suspension of 250 ml. This suspension is placed in a glass-stoppered 250-ml measuring cylinder in which the distance between the 0- and 250-ml graduations is between 20 and 21·5 cm. The cylinder is kept at a thermostatically controlled temperature for the prescribed time; at the end of that time the top nine-tenths of the suspension is drawn off. The content of active agent in the bottom one-tenth is determined and hence that of the top nine-tenths can then be calculated, and the percentage suspensibility determined.

SIZE RANGE—

2 to 76 μ.

REFERENCE—

Collaborative Pesticides Analysis Committee of Europe (C.P.A.C.). Recommended Methods of Analysis for Pesticides, No. 11—"Determination of Suspensibility of Water-dispersible Powders." F.A.O. Plant Protection Bulletin (in the press).

(**26**) W.H.O. METHOD—

This method is similar to the C.P.A.C. method (**25**), except that preparation of the sample by creaming is not included.

SIZE RANGE—

2 to 76 μ.

REFERENCE—

"Specifications of Pesticides," World Health Organisation, Geneva, 1961.

(**27**) MINISTRY OF AGRICULTURE, FISHERIES AND FOOD METHOD—

This method is used in the determination of the particle size distribution of free sulphur in agricultural chemicals. The general principles are as for the Beaker method (**24**), but the sedimentation vessel is a glass tube, 5·5 cm internal diameter and 25 cm long, fitted with a ground-glass stopper at the top and a tube outlet, with a tap, leading from the bottom. A side-tube with a tap is fitted near the bottom of the vessel; this has an upward-pointing nozzle, 1 mm in diameter, on the axis of the tube and 5 cm distant from the bottom. A weighed sample is dispersed in 1 litre of water and sufficient of the resulting suspension is poured into the sedimentation tube up to the graduation mark 20 cm above the top of the side-tube. The suspension is allowed to settle for a specified time at 22° to 25° C; the top 20 cm is run off through the side outlet-tube and its free sulphur content determined by the prescribed method. With a settling time of 166 minutes, the percentage distribution of sulphur particles of 6 μ or less is determined; that of particles of 2 μ or less is given with a settling time of 27 hours.

SIZE RANGE—

2 to 76 μ.

REFERENCE—

Gray, J. R., *J. Sci. Food Agric.*, 1956, **7**, 3.
M.A.F.F., Technical Bulletin No. 1, "Specifications for Pesticides," Third Edition, 1958.

Centrifugal Methods

(28), (29) SHARPLES AND DIETERT CENTRIFUGE METHODS—

The Sharples instrument is a supercentrifuge that has been adapted for very small particles, such as tobacco-mosaic virus (*i.e.*, approaching molecular dimensions).

The Dietert instrument is a centrifugal classifier useful for production control of powdered materials when extremely small particles are involved.

SIZE RANGE—

0·05 to 50 μ.

REFERENCE—

Schachmann, H. K., *J. Phys. Colloid Chem.*, 1948, **52**, 1034.

(30) KAYE DISC CENTRIFUGE—

In this technique particles are spun in a centrifuge in a cylindrical transparent tank in which there is an entry port. The centrifuge is run up to speed partially filled with clear liquid. When steady-state conditions are attained the free surface of the fluid is in effect cylindrical, the axis of the surface being coincident with the axis of rotation. The powder to be examined is injected as a low-concentration suspension through the entry port, and a layer of suspension is formed on top of the free surface of the fluid in the tank. The particles travel outwards, and the concentration of each size is measured by means of a light beam passing through the tank at a given distance from the centre of rotation.

The equipment is essentially a photosedimentometer employing centrifugal force to aid the sedimentation of fine particles; it is therefore subject to the limitations of the photosedimentometer theory.

SIZE RANGE—

1 to 50 μ.

REFERENCE—

Kaye B. H., Ph.D. Thesis, London University; British Patent No. 895,222, 1962.

ELUTRIATION PROCESSES

Elutriation differs from sedimentation in that fluid moves vertically upwards and thereby carries with it all particles whose settling velocity by gravity is less than the fluid velocity. In practice, complications are introduced by such factors as the non-uniformity of the fluid velocity across a section of an elutriating tube, the influence of the walls of the tube and the effect of eddies in the flow. In consequence, any assumption that the separated particle size corresponds to the mean velocity of fluid flow is only approximately true; it would also require an infinite time to effect complete separation.

Stokes's law cannot always be used in the calculations, since laminar flow rates are often exceeded.

Gravitational Methods

(31) ANDREWS ELUTRIATOR—

This is a form of elutriator for use with liquid flow. It consists of a small-diameter and a large-diameter vessel in series. The larger vessel contains a circulating device for breaking down aggregations of powder. Elutriation produces three grades of particles, one collected in the first vessel, one in the second and the other in the overflow.

SIZE RANGE—

10 to 76 μ.

REFERENCE—

Andrews, L., *Proc. Inst. Eng. Inspection*, 1927/1928, p. 25.

(**32**) BLYTHE ELUTRIATOR—

The instrument consists of six beakers almost filled with water and connected in series by siphons; the diameter of the up-going tube of each siphon is $\sqrt{2}$ times that of the preceding one. Each of the beakers is mechanically stirred, and water from a constant-head tank flows at a controlled rate through the system.

A dispersed powder is washed into the first beaker, where the coarsest particles are retained. All particles below a specific size, determined by the water velocity up the first siphon, are carried over to the second beaker. The entraining velocity in the second siphon is only half that of the first so that, again, the coarser particles remain in the second beaker and the finer ones are carried along. It is thus possible to separate into seven grades. Though the process is lengthy, no attention is needed.

SIZE RANGE—

10 to 60 μ.

REFERENCE—

Pryor, E. J., Blythe, H. N., and Eldridge, A., "Recent Developments in Mineral Dressing," *Inst. Min. Met.*, 1953, 11.

(**33**) GONELL AIR ELUTRIATOR—

This consists of a cylindrical brass tube (or a series of tubes) with a conical base. An air inlet is provided in this base on the axis of the tube. The sample of powder is placed in the inlet cone, and air is blown through the largest tube until separation is deemed complete, or for specified periods of time if working to the B.S. specification. The residue is removed, weighed, and transferred to a smaller-diameter tube, and the test is repeated. The tubes should have polished internal surfaces and should be periodically tapped or vibrated to disturb settled dust. Weight of powder required, about 1 g. Time for analysis at least 4 hours.

SIZE RANGE—

5 to 76 μ.

REFERENCE—

British Standard 893 : 1940. Method of testing dust extraction plant.

(**34**) MODIFIED GONELL ELUTRIATOR (FUEL RESEARCH STATION APPARATUS)—

The shape of the dust reservoir was modified in the Gonell apparatus (**33**), and specified in B.S. 893; the changes are described by Hughes. It is suggested that elutriation should be started by an initial high-velocity blast sufficient to carry all the dust into the elutriating tube, but of such short duration that the particles do not have time to reach the top of the elutriating tube before the velocity falls to normal.

SIZE RANGE—

5 to 76 μ.

REFERENCES—

British Standard 893 : 1940. Method of testing dust extraction plant.
Hughes, T. H., *Engineer* (*Lond.*), 1957, **203**, 860.

(**35**) ROLLER AIR ELUTRIATOR—

This is a proprietary instrument of American origin. The special feature is a swan-necked tube through which air is admitted to the elutriator tube. This is vibrated and has a nozzle so designed that the powder contained in the entry tube is continuously circulated to break down agglomerations. The sample weight required is about 10 g.

The general operating procedure follows that for the Gonell elutriator.

SIZE RANGE—

5 to 76 μ.

REFERENCES—

A.S.T.M. Specification No. B.293–54T; 1954.
British Standard 3406 : Part 3 : 1962.

(36) MINIATURE ELUTRIATOR—

This apparatus is not a general-purpose instrument, but was developed by Imperial Chemical Industries Ltd. for the rapid elutriation of powders that are difficult to disperse, and in the size range 10 to 76 μ. Samples of 0·1 to 0·5 g are used. The special feature is the entrainment of the powder by a high-velocity air jet, which blows downwards into a thimble containing the powder at the base of the elutriating tube.

The elutriating tube is smaller than that of other elutriators; it is 14 inches long and 1 inch in diameter. The general operating procedure follows that for other elutriators.

SIZE RANGE—

10 to 76 μ.

REFERENCES—

Stairmand, C. J., *Engineering*, 1951, May 18th.
British Standard 3406 : Part 3 : 1962.

(37) HAULTAIN ELUTRIATOR, INFRASIZER—

This elutriator was originally intended for mining and metallurgical laboratories in Canada. It consists of six conical elutriating tubes in series, air flow entry being to the smallest. Air enters through a conical seating supporting a golf ball, which, by rotation and impact, breaks down agglomerations of particles. The general operating procedure follows that for other elutriators.

SIZE RANGE—

5 to 200 μ.

REFERENCES—

Haultain, H. E. T., *Trans. Can. Min. Met.*, 1937, **40**, 229.
Price, E. W., *Ind. Eng. Chem.* (*Process Design & Development*), 1962, **1**, 79.

Centrifugal Methods

(38) BAHCO CENTRIFUGAL DUST CLASSIFIER—

This is a proprietary instrument that is essentially a centrifugal air elutriator. Air and dispersed dust samples are drawn through the cavity of a rotating hollow disc in a radially inward direction against centrifugal forces. The dust particles are thus divided into under- and over-size fractions, collected, and weighed. Separation into different size-fractions is made by altering the air velocity. About 20 g of dust are required for the sample, and 8 size determinations can be made in 2 hours.

SIZE RANGE—

5 to 100 μ.

REFERENCE—

Berns, E. G., *Brit. J. Appl. Phys.*, Supplement No. 3, 1954, S208.

(39) B.C.U.R.A. CENTRIFUGAL ELUTRIATOR—

This consists of two parallel concentric discs of equal radius mounted on a motor-driven spindle within a short cylindrical co-axial chamber. The discs are spun at a controlled rate and air is drawn through the system, also at a controlled rate. The powder entrained in this air enters the chamber axially, flows outwards over the surface of the first disc, passes between the two from the periphery to the centre and leaves axially. The air and dust pick up tangential velocity and between the discs the dust is acted upon by two forces, centrifugal outwards and drag inwards. Coarse particles are collected from the circumference of the chamber, fine particles pass into the exit. There is appreciable turbulence between the periphery of the discs and the chamber wall, so that considerable scrubbing takes place, the coarse particles being cleaned of fines. The cut size can be calculated, but, because the flow is not laminar and uniform, the cut size differs from the theoretical by a constant that has to be determined empirically.

SIZE RANGE—

5 to 40 μ.

REFERENCE—

Godridge, A. M., Badzioch, S., and Hawksley, P. G. W., *J. Sci. Instrum.*, 1962, **13**, 611.

Winnowing Methods

The principle of these methods is to allow the powder particles to fall vertically (if gravity is used) in a stream of winnowing fluid moving horizontally. The particles therefore move under two velocities, a horizontal one equal to the fluid speed, and a vertical one depending on the particle size. The particles are thus sorted out along the floor of the winnowing chamber, the larger (or heavier) being carried least and the smaller the greatest distance.

The devices are often used, by suitable adjustment of the fluid viscosity and floor length, for carrying out a preliminary sorting of the coarse from finer particles. They may also be used for direct sizing of airborne particles without the need for intermediate collection with the associated dangers of aggregation or breakdown.

(40) TIMBRELL SAMPLER—

In this apparatus, clean winnowing air is passed along a horizontal tube, cleaned, and recirculated. The dust particles, entrained in air, are drawn in through a narrow tube and liberated at one end of the horizontal tube; they are subsequently deposited along the floor of the tube according to their free-falling speeds. The apparatus is first calibrated by using glass spheres and examining the slide produced microscopically, thus achieving a curve for Stokes's diameter *versus* distance travelled.

If all that is required is a curve of frequency *versus* Stokes's diameter, this may now be determined on the sample dust, counting at various distances with a microscope, or by use of a densitometer. However, it is also possible to study the variation of projected diameter (by using a microscope with eyepiece graticule) with Stokes's diameter in the sample, and the extent of true aggregation is obviously simple to determine.

If the sampling is carried out for a longer period it is possible to obtain a weight-distribution curve.

The apparatus may be modified to give intermittent sampling of a continuous stream, such as flue gases.

An excellent review, with references to original work, has been made by Timbrell.

SIZE RANGE—

2 to 15 μ.

REFERENCE—

Timbrell, V., *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S12 and S86.

(41) WALTON HORIZONTAL ELUTRIATOR—

This consists of a short horizontal duct containing horizontal shelves, thus producing a stack of low horizontal passages.

The back of the duct is covered by a filter-paper. Air is drawn into the duct, and the larger particles of dust are deposited on the floors of each of the passages, the fine dust being caught by the filter-paper.

The basic principles were described by Walton, and the commercial instrument, which differs slightly from the original design, is known as the Hexlet Collector.

The design is used particularly for pneumoconiosis work, the front of the instrument simulating the nasal passages and the rear retaining particles similar to those found in the lungs.

SIZE RANGE—

5 to 76 μ.

REFERENCE—

Walton, W. H., "Horizontal Elutriators," part of a paper entitled "Theory of Size Classification of Airborne Dust Clouds by Elutriation," *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S303.

(42) CONIFUGE—

This apparatus is essentially a conical self-pumping centrifuge, into which the air containing the sample of dust is drawn at the rate of 25 ml per minute and subjected to centrifugal force and the "winnowing" effect of the clean air recycled within the apparatus. Since the two forces acting on the particle are at an angle to one another, the resulting deposit takes the form of a "spectrum," which can then be counted by one of the microscope techniques or determined photodensitometrically.

The method is applicable to airborne particles of any type or shape, and is independent of whether the particle is solid or liquid. The apparatus is readily calibrated with solid spheres of known density, *e.g.*, paraffin wax. Alternatively, Stokes's law may be used to effect the calibration.

SIZE RANGE—

0·5 to 30 μ for spherical particles of unit density.

REFERENCE—

Sawyer, K. F., and Walton, W. H., *J. Sci. Instrum.*, 1950, **27**, 272.

(43) CONICYCLE—

This consists of a pair of concentric rotating cylinders. At each end are two discs, one closing the ends of the inner cylinder, the other being separated from the first by an annular space. At the inlet end of the instrument, where the end disc has the same diameter as the outer cylinder, an extension of the outer cylinder forms a disc baffle almost to the axis, and divides the annulus so that air passes first towards the axis, then under the baffle and outwards to reach the space between the two cylinders. At the exit end, the end disc extends to a greater radius than the outer cylinder. When the instrument is rotating at high speed (8000 r.p.m.) the difference in radii of the two end annuli causes the instrument to act as a centrifugal fan driving air out of the exit. Centrifugal forces on the particles entering the inlet oppose drag forces pulling them in, so that only particles less than a specific size (usually 10 μ) enter the instrument. The particles that enter are deposited according to size along the inner wall of the outer cylinder, except for a fraction of fine particles (less than 1 μ) that escape into the exit.

The instrument was designed to simulate the action of the human body, the retained particles being similar to those captured by the lungs.

SIZE RANGE—

1 to 10 μ.

REFERENCE—

Carver, J., Nagelschmidt, G., Roach, S. A., Rossiter, C. E., and Wolff, H. S., *Min. Eng.*, 1962, **121**, 601.

(44) CASCADE IMPACTOR—

Particle-laden air is caused to pass through a series of graded jets or slits, one after another in order of size, the largest aperture being the first. Facing each jet is a glass slide, or similar collecting device. It is usual to employ four stages, but as many as seven have been used. The lower limit of particle size that can be captured depends on the velocity of the suspension through the orifice and an absolute limit is reached when this velocity becomes that of sound. The slides can be examined in the usual manner.

SIZE RANGE—

0·7 to 20 μ.

REFERENCES—

Gillespie, T., *J. Colloid Sci.*, 1955, 266.
Gillespie, T., and Rideal, E., *Ibid.*, 1955, 281.
Laskin, S., U.S. Atomic Energy Commission Report MDDC-1500, Oak Ridge ,Tenn., 1946.
May, K. R., *J. Sci. Instrum.*, 1945, **22**, 187.
Orr, C., jun., and DallaValle, J. M., "Fine Particle Measurement," The Macmillan Co., New York, 1959.
Pilcher, J. M., Proceedings of the 42nd Annual Meeting, Chemical Specialities Manufacturing Association Inc., New York, December, 1955.
Ranz, W. E., and Wong, J. B., *A.M.A. Arch. Ind. Health*, 1952, **5**, 464.
——, ——, *Ind. Eng. Chem.*, 1952, **44**, 1371.
Wilcox, J. D., *A.M.A. Arch. Ind. Hyg.*, 1953, **7**, 376.

A.II. IMAGE FORMATION

In methods involving image formation there is a common factor. Radiation normally propagated in rectilinear fashion, such as light or an electron beam, is interrupted by the particles under examination, and the pattern of the interruption can be observed in different ways, *e.g.*, optically or photo-electrically.

Light Beam: (a) Optical Microscope

The image produced may be viewed either directly by the eye or projected on to a screen. The latter method is usually the more convenient and involves less eyestrain; it is less satisfactory than direct observation for fine sizes near the limit of optical resolution. Sizing is commonly achieved by comparison of the particle images with a scale or graticule. Since the process is tedious, sizing is sometimes performed automatically.

The smallest resolvable particle size is a function of the wavelength of the light used and may be about 0·5 to 1 μ.

Individual Sizing and Counting—

(**45**) GRATICULES—

The principle used is to observe the projected image of the particles formed in the eyepiece of a microscope and to compare their sizes with standard accurately drawn shapes held in the same plane.

The earliest graticule for use in this type of work was designed by Patterson and Cawood[1] in 1936. It consists of a series of solid "globes" and lined "circles" of graded diameters aligned at the top and bottom of a series of counting rectangles. The size of the particle can be determined by assessing whether it is larger, and protrudes behind a globe of given diameter, or is smaller than a circle of the same diameter.

A modification of this basic graticule was described by Fairs[2] in 1943. In this, the globes and circles were in a geometrical progression based on a $\sqrt{2}$ difference in diameter. This he considered to be the finest distinction that the eye can perceive, and it permits counts to be made at various magnifications to overlap in identical size ranges. Fairs also described two other graticules intended for use with larger particles. One of these contains three globes and circles in geometric progression and one larger circle in the centre. The other contains five circles arranged like a shooting target centred on a cross-wire.

A further modification, usually referred to as the Porton Graticule, was described by May[3] in 1945. This is essentially similar to Patterson and Cawood's graticule, except that the diameters of the globes and circles increase in the $\sqrt{2}$ progression and this is continued in the right-hand portion of the counting rectangle by a series of ruled lines, thus extending the range by using only one magnification. It is most useful when sizing circular particles, such as droplets or stains left by droplets.

Various other modifications have been described in the literature, notably, one described by Watson[4] in 1951, which is a much simplified version of the Fairs graticule for use by inexperienced workers.

All graticules are used in the same manner. The particles are deposited as evenly as possible on a glass microscope slide, either by use of a thermal precipitator or by making a smear, an inert liquid being used as carrier. The slide is then viewed at any suitable magnification and the image so formed compared with the image of the graticule, which is held in the eyepiece. The graticule may be previously calibrated by use of a stage micrometer. Several counts can be made at various magnifications and so a considerable size range may be covered.

For simple circular particles an eyepiece micrometer may be used instead of a graticule. For irregularly shaped particles, a globe and circle graticle is almost essential. If the particles are long and rod-like, an eyepiece micrometer may be used to find their dimensions, but the projected area, as measured by the globe and circle graticule, is no longer meaningful.

Since considerable experience is required in the use of these graticules, Watson[5] has designed a test strip consisting of 100 numbered irregular particles embedded in a standard 3-inch by 1-inch microscope slide. This is now commercially available. It may be used to train workers, and should also be used to give frequent checks on workers already engaged on routine counting work, as the judgment required in making an assessment will not only vary from worker to worker, but may vary with time for any given worker. Despite these difficulties, reasonable reproducibility may be achieved with practice.

SIZE RANGE—

1 μ upwards.

REFERENCES—

1. Patterson, H. S., and Cawood, W., *Trans. Faraday Soc.*, 1936, **32**, 1084.
2. Fairs, G. L., *Chem. & Ind.*, 1943, **57**, 174.
3. May, K. R., *J. Sci. Instrum.*, 1945, **10**, 187.
4. Watson, H. H., *Brit. J. Indust. Med.*, 1951, **9**, 80.
5. ——, *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S101.

British Standard 3406 : Part 4 : 1962, p. 196.

(46) KONIMETER (CONIMETER)—

A screened sample is aspirated and allowed to impinge on to a surface. The sample is then positioned under a microscope fitted with a special grid micrometer (graticule).

In the Zeiss Konimeter, the operation of aspiration and examination are performed in the same instrument by means of a rotatable stage.

SIZE RANGE—

1 μ upwards.

REFERENCE—

Kotze, R., Miners' Phthisis Prevention Committee: Final Report. Union of South Africa, Johannesburg, January 10th, 1919.

(47) ZEISS-ENDTER PARTICLE SIZE ANALYZER—

This equipment is a semi-automatic device for microscope counting. The microscope slide or photomicrograph is examined with a spot of light adjustable in size by an iris diaphragm. The spot of light is centred on a particle, the diaphragm adjusted to the size of the particle and a count of the size is then carried out automatically by depressing a foot-switch. This technique is quicker than manual counting and overcomes the difficulties of visually comparing particles with fixed circles some distance away.

SIZE RANGE—

1 μ upwards.

REFERENCE—

Endter, F., and Gebauer, H., *Optik*, 1956, **13**, 97.

Automatic Sizing and Counting—

(48) CASELLA INSTRUMENT—

The method assumes uniformly opaque material—although some variation can in practice be tolerated—and a fairly low (say, less than 4 per cent. by area) particle concentration on the slide is necessary. A determination takes ½ to 1½ hours, depending on the material and on the number of size classes assessed.

A magnified image of the particle plane is moved past a slit perpendicularly to its length. The light passing through the slit falls on a photomultiplier cell, which emits a signal pulse each time the slit is crossed by a particle, the pulse amplitude depending on the maximum light obscuration produced by the particle as it crosses. The pulses are discriminated at five different size levels simultaneously, and their numbers recorded by scaling units. From the counts at a number of different slit lengths the concentration and sizes of particles can be deduced. The method is referred to as "wide-track statistical scanning."

The metal shadowing technique offers a means of sizing material in which there is too wide an opacity variation for direct counting to be carried out. The instrument is less flexible than a visual observer in regard to the area of the slide sampled, but more flexible in regard to the spacing of the size classes.

SIZE RANGE—

2 to 50 μ.

REFERENCE—

Hawksley, P. G. W., *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S125.
Hawksley, P. G. W., Blackett, J. H., and Fitzsimmons, A. E., *Ibid.*, 1954, Supplement No. 3, S165.
Morgan, B. B., and Meyer, E. W., *J. Sci. Instrum.*, 1959, **36**, 492.

(**49**) TIMBRELL INSTRUMENT—

This instrument utilises the principle of double-image microscopy, and comprises a special microscope and an associated electrically operated particle-size analyser. The apparatus can be connected to digital read-out and print-out on tape facilities for processing of the results by computer.

SIZE RANGE—

1 to 25 μ.

REFERENCE—

Timbrell, V., *Nature*, 1952, **170**, 318.
Barnett, M. I., and Timbrell, V., *Pharm. J.*, 1962, 379.

Light Beam: (b) Flying-spot Principle

The difference between this principle and that of the optical microscope methods is that the whole field is not illuminated simultaneously, but is scanned by a fine light spot. Interruption of the illumination is measured electronically.

(**50**) RANK CINTEL INSTRUMENT—

A moving spot of light, produced on the face of a cathode-ray tube, is focussed on the sample by means of a suitable lens system. The amount of light passing through or reflected from the sample varies according to the optical density and configuration of the individual particles of the sample. These changes in light intensity are detected by a photomultiplier, the signal from which is fed into the counting and sizing circuits. A memory device prevents each particle of the sample being counted more than once, and sizing is accomplished by pulse subtraction.

The method requires at least a 10 per cent. difference in contrast between particle and background. For maximum accuracy the particles should have well-defined edges, be regular in shape and their concentration should be low to avoid overlapping. The particles may be dark on a light background, or *vice versa*; they may be measured on a support (microscope slide, metal plate, glass cell, paper) or they may be measured from photographic negatives. The time taken to analyse a given sample depends on the particle density of the sample and its degree of heterogeneity; it is usually about 10 to 60 minutes. The total projected area of the particles in the sample and of an average particle may be measured in 2 to 20 minutes.

SIZE RANGE—

1 μ upwards by using transmitted light;
20 μ upwards by using reflected light from opaque specimens.

REFERENCES—

Taylor, W. K., *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S173.
Causley, D., and Young, J. Z., *Research*, 1955, **8**, 430.
Furmidge, C. G. L., *Brit. J. Appl. Phys.*, 1961, **12**, 268.

(**51**) MULLARD INSTRUMENT—

The basic principles of scanning, counting and sizing are similar to the Rank Cintel instrument (**50**). The main difference is that this instrument operates only for samples photographed on 35-mm film. A wide range of particle sizes may be measured by varying the magnification in the preliminary photographic process.

SIZE RANGE—

1 μ upwards by using transmitted light;
20 μ upwards by using reflected light from opaque specimens.

Electron Beam

(**52**) ELECTRON MICROSCOPE—

The electron microscope has much greater magnifying and resolving powers than has the optical microscope. It is used to produce a photographic image of a sample, the photograph being subsequently examined by normal optical methods. Special precautions are necessary for preparing the sample and for calibrating the magnification.

SIZE RANGE—

0·001 to 20 μ.

REFERENCE—

Cartwright, J., *Brit. J. Appl. Phys.*, 1954, Supplement No. 3, S109.

A.III. SCATTERING OF RADIATION

Light Beam

The intensity of light scattered from a parallel beam by an array of particles varies with direction and is dependent on the ratio λ/D, where λ is the wavelength of the incident radiation and D is the diameter of the particle. It also depends critically on the refractive indices of the materials considered. A comprehensive review on the relevant theory has been given by Van de Hulst.[1] Several techniques have been developed for special systems that permit an estimate to be made of the particle size distribution and size from a study of the scattered radiation.

(53) GOULDEN LIGHT SCATTERING TECHNIQUE—

This method[2] has been developed for measuring the size of fat globules in milk.

SIZE RANGE—

0·01 to 100 μ.

REFERENCES—

1. Van de Hulst, H. C., "Light Scattering by Small Particles," John Wiley & Sons Ltd., London, 1957
2. Goulden, J., *Brit. J. Appl. Phys.*, 1961, **12**, 456.

X-ray Beam

(54) X-RAY METHODS INVOLVING LOW-ANGLE SCATTERING TECHNIQUES—

In ordinary X-ray diffraction work, some information in respect of crystallite size or grain size can be obtained from line-broadening effects. Diffraction cameras, however, are mainly used for the study of crystalline substances.

The type of light scattering referred to in A.IV (Young's rings), however, also occurs when X-rays are scattered by a dispersion of small particles. Since X-rays are of extremely short wavelength, the scattering occurs with small particles in the size range of about 0·002 to 0·1 μ (about 20 to 1000 Å) and it reaches a maximum in directions that make angles of only a few degrees with the forward direction of the X-radiation.

Special cameras have been designed to gain the maximum amount of information about this forward scatter. The methods may be equally well applied to both crystalline and amorphous substances. In contrast to ordinary diffraction cameras, long specimen to film distances are used, and special precautions are taken in the design of the collimeter and slit system to reduce instrumental scatter. Details of different designs and results of tests (including comparision with standard methods) are given in the references below.

SIZE RANGE—

0·002 to 0·1 μ.

REFERENCES—

Gerold, V., "Small-angle Scattering and Its Use in Particle-size Determination," *Z. angew. Phys.*, 1957, **9**, 43.
Kratley, P., *Kolloid-Z.*, 1955, **144**, 110.
Guinier, A., *Ann. Phys.*, 1939, **12**, 161.
Riley, D. P., "X-ray Diffraction by Polycrystalline Materials," *Inst. Phys.*, London, 1955.

A.IV. DIFFRACTION OF RADIATION

(55) YOUNG'S DIFFRACTION RINGS—

If a point source of monochromatic light, wavelength λ, is viewed through a screen of spherical particles with uniform diameter, d, the image is surrounded by a system of alternately light and dark diffraction rings. The dark rings are identified as first order, second order, . . . nth order, by counting outwards from the source image.

If θ is the angle subtended at the point of observation by the mean semi-diameter of the ring of *n*th order, the following relation exists—

$$d = \frac{n + 0{\cdot}22\lambda}{\sin \theta}$$

Particles with an acceptable degree of uniformity are vegetable spores, blood cells and mist droplets. The size of mist droplets is conveniently estimated by measuring the diameters of the minima between lunar haloes. For example, if the apparent diameter of the first order dark ring is five times that of the lunar disc (31′ 7″), $\sin \theta = 0{\cdot}02262$. If the mean wavelength of visible light is taken as $\lambda = 0{\cdot}57\ \mu$, d is $31\ \mu$.

SIZE RANGE—

5 to 50 μ.

REFERENCE—

Humphreys, H., "Physics of the Air," McGraw-Hill Book Co., New York and London.

A.V. ELECTRICAL PROPERTIES

(56) CONDUCTIVITY (COULTER COUNTER)—

This commercially available equipment utilises the change in resistance of the system caused by the presence of a particle in an electrolyte. The suspension flows through a small aperture having an immersed electrode on either side, with particle concentration such that the particles traverse the aperture substantially one at a time. Each particle passage displaces electrolyte within the aperture, momentarily changing the resistance between the electrodes and producing a voltage pulse of magnitude proportional to the particle volume. The resulting series of pulses is electronically amplified, scaled and counted.

SIZE RANGE—

0·2 to 300 μ.

REFERENCE—

Kubitschek, J., *Research*, 1960, **13**, 129.

A.VI. SIEVES

Woven Wire Sieves

Sizing by sifting, although simple in conception, has many practical and theoretical difficulties. The technique is covered by British and foreign standard specifications.

(57) BRITISH STANDARD SPECIFICATIONS—

British Standard 410 gives details of sieve construction. Part 1 specifies requirements for the construction of fine and medium test sieves; Part 2 refers to the wire cloth for the test sieves and includes tables of aperture sizes, wire diameters, screening areas and aperture tolerances; Part 3 specifies requirements for coarse test sieves; Part 4 deals with the construction of the perforated plates for coarse sieves. Appendices cover methods of examination, measurement and calibration of sieves, and particulars of the American (A.S.T.M.), German (D.I.N.) and Institute of Mining and Metallurgy (I.M.M.) standard sieve sizes.

The British specification covers two grades of wire sieves—the normal sieves with tolerances from ±9 per cent. to ±3 per cent., and the special sieves with tolerances from ±7 per cent. to ±2 per cent.

PARTICLE-SIZE RANGE—

76 to 3353 μ (exceptionally, 53 to 3353 μ).

British Standard 1796 contains sections dealing with principles of sifting, equipment to be used, preliminary preparation of the sample, weight of samples to be used in tests, use of wet and dry methods for the preliminary removal of fine dust, hand sifting to the "end-point" by the rate test, machine sifting to the "end-point" by the rate test, loss of dust during sifting, sifting tests on materials with only a small amount of oversize on the sieve and routine sifting tests. Information is also given on the correct method of reporting results and limits of accuracy of the results obtained. Appendices give methods of subdividing bulk samples, and notes on sifting procedure.

Corresponding American, Dutch, French and German standard specifications are available: these are listed below, with the particle-size ranges catered for—

U.S.A.	A.S.T.M. E-11/39 (Fine series)	37 to 5660 μ
Netherlands	N 480	50 to 850 μ
France	AFNOR NF X11-501-1938	40 to 5000 μ
Germany	DIN 1171	60 to 5000 μ

Recent Techniques

(Not yet standardised)

(58) ELECTROFORMED SIEVES—

Fine-mesh precision sieves formed of nickel by a photograving and electroplating technique are available at a few aperture sizes down to 20 μ. The regularity of aperture size is good (one measurement of a nominally 20 μ aperture sieve gave 97·5 per cent. of the apertures in the range 20 ± 1·5 μ, none having an error greater than ±2·5 μ), but the sides of the apertures are less smooth than those of woven wire sieves. Nothing is known of the expected life of such sieves.

SIZE RANGE—

16 to 53 μ.

REFERENCE—

Daeschner, H. W., A.S.T.M. Symposium, 1959, *Special Technical Publication No.* 234, American Society for Testing Materials, Washington.

(59) AIR-JET SIFTING—

The Alpine "Air-Jet Sieving Machine" utilises the principle of a rotating jet blowing air up from beneath an enclosed sieve. This keeps the powder moving on the sieve while the undersize particles are drawn through the rest of the sieve by the air returning, *via* a suitable filter, to the suction side of a fan.

SIZE RANGE—

53 to 3353 μ.

REFERENCE—

Lauer, O., *Staub*, 1960, **20**, 69.

B.I. PERMEABILITY TO FLUID FLOW

The relevance of methods in this section to the measurement of specific surface depends on certain relationships that are assumed to hold between the rate of fluid flow, the pressure head, viscosity of the fluid, density and specific surface of the powder and porosity of the powder bed. Generally, the relationship assumed is the Kozeny equation or some modification of it.

It must be stressed that permeability of the bed is the property that is actually measured; the validity of the estimates of specific surface derived therefrom depends on the validity of the relationships assumed. The methods give useful comparisions between different samples of materials of the same type, and hence are frequently used in control of raw materials. Anomalous results may be obtained if materials with widely divergent size or shape distributions are compared; the results of permeability measurements should therefore be interpreted with due caution. Some preparation of the sample, such as removal of coarse particles by sifting, may be necessary to achieve reproducible results.

REFERENCES—

Kozeny, J., *Sitzber. Akad. Wiss. Wien. Math-naturw. Kl.*, 1927, **IIa**, 271.
Rigden, P. J., *J. Soc. Chem. Ind.*, 1947, **66**, 130T.

Liquid Flow

(60) CARMAN METHOD—

A liquid is allowed to flow, under constant pressure head, through a bed of the powder in a cylindrical tube. The rate of flow, pressure head, viscosity of the liquid, density of the powder and porosity of the bed are measured; the specific surface of the powder may then be calculated. In its original form, the apparatus was applicable to corase-grained powders; subsequent modifications, by increasing the pressure, are stated to have extended the range down to 2 to 3 μ. This lower limit corresponds to a specific surface of about 10,000 sq. cm per g.

REFERENCES—

Carman, P. C., *Trans. Inst. Chem. Eng.*, 1937, **15**, 150;
J. Soc. Chem. Ind., 1938, **57**, 225 and 1939, **58**, 1;
Disc. Faraday Soc., 1948, **3**, 72.

Gas Flow

(**61**) LEA AND NURSE METHOD—

The apparatus consists of a cell, a manometer and a flowmeter. The powder is compacted into a bed in the cell and dry air is allowed to flow through it under a constant pressure differential, the rate of flow being measured. A series of measurements is made at several pressure differentials and the gradient of the pressure-flow graph is determined. Thence, from the density of the powder and the porosity of the bed, the specific surface may be calculated in accordance with the Kozeny - Carman equation.

REFERENCES—

Lea, F. M., and Nurse, R. W., *J. Soc. Chem. Ind.*, 1939, **58**, 278.
British Standards 12 : 1958 and 915 : 1947.

(**62**) GOODEN AND SMITH METHOD—

The principle is the same as that of the Lea and Nurse method (**61**). It differs in having a fixed pressure on one side of the bed. Since the air also passes through a resistance flowmeter, the pressure on the downstream side of the bed varies with the rate of flow. When the manometer across the flowmeter becomes steady, the reading is taken and is used to obtain an "average particle size." An automatic calculator is provided with the instrument.

REFERENCE—

Gooden, E. L., and Smith, C. M., *Ind. Eng. Chem.*, *Anal. Ed.*, 1940, **12**, 479.

(**63**) FISHER SUB-SIEVE SIZER—

In this instrument, which is developed from the Gooden and Smith method (**62**), a standardised compaction procedure is used. The surface area is read directly from one of a family of curves on a chart incorporated in the instrument.

REFERENCE—

Fisher Scientific Company, Pittsburg, Pa., U.S.A.; Kek Ltd., Ancoats, Manchester 12.

(**64**) RIGDEN METHOD—

The powder is compacted into a bed in a B.S. 12 cell, and the two ends of the cell are connected to the two ends of a U-tube containing an oil of low vapour pressure. The oil is displaced initially by drawing up on one side; as it returns to its equilibrium level it forces air through the powder bed. The pressure differential thus diminishes as flow proceeds. The time for the oil to travel between two marks on the U-tube limb is measured and the specific surface then calculated.

REFERENCE—

Rigden, P. J., *J. Soc. Chem. Ind.*, 1943, **62**, 62 and 1947, **66**, 130T.

(**65**) GRIFFIN SURFACE AREA APPARATUS—

This apparatus is based on the work of Rigden (**64**). The oil in a U-tube is displaced and, in returning to equilibrium, forces air through a powder bed. The manufacturers recommend that the bed be formed by compacting four or five successive scoop samples with a plunger. With the addition of a 0- to 760-mm mercury manometer, the rate of flow at reduced pressures can be used to give the mean pore radius. On a sample of Portland cement, a spread of ±2 per cent. on the mean is claimed.

(66) BLAINE FINENESS TESTER—

The principle is the same as that of the Rigden method (**64**). The powder is contained in a cylindrical cell, the lower end of which is connected to a U-tube manometer. Provision is made for the withdrawal of air from the space between cell and manometer, so that the level of oil in the manometer tube is raised. The time for the manometer liquid to travel between two lines on the tube is then measured. The method is therefore of the diminishing-pressure-differential type.

REFERENCE—

Blaine, R. L., *A.S.T.M. Bulletin*, 1943, No. 123, 51;
A.S.T.M. Standards, 1958, Part 4, C204–55, p. 140.

(67) SPILLANE METHOD—

The principle is the same as that of the Rigden method (**64**). In effect, it gives a direct pointer reading of the time taken for mercury to travel between two points on a manometer tube. The dial may be calibrated, by means of samples already tested by the Rigden or Lea and Nurse methods, to give direct readings of the specific surface of powders of uniform specific gravity.

SPECIFIC SURFACE—

1500 to 4500 sq. cm per g.

REFERENCE—

Spillane, F. J., *Analyst*, 1957, **82**, 712.

B.II. ABSORPTION METHODS

There are various methods for the determination of the surface area of solids based on the adsorption of a mono- or polymolecular layer on the surface of the solid. These methods do not measure the particle diameter or projected area as such, but measure the available surface per gram or millilitre of powder. With catalysts and adsorbent materials this is more relevant than particle size.

Static: (a) Absolute Method

(68) HARKINS AND JURA (ABSOLUTE METHOD)—

In this method[1] (designated HJa), the clean, de-gassed, finely-powdered solid is kept in the saturated vapour of a liquid that wets it. The solid adsorbs a polymolecular film whose outer surface, at equilibrium, has the same total surface energy, h_l, as the liquid itself. The powder is then dropped into the same liquid in a calorimeter to obtain a measure of the heat developed by the disappearance of the adsorbed film. If water is chosen as the liquid, the energy change involved is fixed at 118·5 ergs per sq. cm at 95° C. The low energy available necessitates extremely accurate calorimetry, and measurement of temperature to ±0·00002° C is required. Extremely constant temperature control is also essential and, unless the specific surface is of the order of at least 3 to 9 sq. m per ml, high accuracy is not attainable.

The method has been used to give an extremely accurate determination of surface area of reference samples; a sample of anatase with a surface area of 13·8 sq. m per g, determined by this method, is used in many laboratories for calibration purposes for other methods.

Static: (b) Involving use of Adsorption Isotherms

There are two main methods of determining surface area involving the use of absorption isotherms. Both methods make use of a similar technique, but differ mainly in the treatment of results.

(69) BRUNAUER, EMMETT AND TELLER—

In this method[2,3] (designated BET), the surface area is not measured directly, but the number of molecules of the adsorbed substance required to give a monolayer (N) is determined; if the mean area per molecule (σ) of the adsorbed substance is known by other means, the area of the solid may be calculated as—

$$\epsilon = N\sigma.$$

(**70**) HARKINS AND JURA—

In this second method of Harkins and Jura[4,5] (designated HJr), the surface area is given directly after standardisation by the absolute method (**68**).

Determination of Isotherm—

Since both the "BET" (**69**) and "HJr" (**70**) methods use the adsorption isotherm, the experimental details of determining this are summarised as follows, and references are given for the treatment of results.

The powder under investigation is thoroughly de-gassed in a vacuum and small amounts of gas or vapour are added at constant temperature; the resultant pressure is noted. With no solid present, the pressure would follow the gas laws; with powder present, the pressure is lower. A curve of volume of gas admitted *versus* pressure may be produced and, from this isotherm, the surface area may be determined by either method.

Adsorption from Solution—

A solid will adsorb a surface-active material or a dyestuff from solution and an isotherm may be constructed, use being made of concentration of adsorbate instead of pressure. Calibration with standards is essential. This method is limited in its application because solids soluble in the solvent cannot be used; further, sometimes (*e.g.*, with electrolytes on charcoal or silica gel) the solvent may be adsorbed to a greater extent than the solute, producing apparent negative adsorption. The adsorption is generally believed to be a consequence of the solid presenting a solid - liquid interface that can accumulate a surface-active compound in the same way as can an air - liquid interface. The degree of adsorption increases with surface tension, so that water is usually used. As an example, a dyestuff, initially adsorbed from water, may often be removed from charcoal by alcohol or acetone.

Modifications for Control Purposes—

The basic methods may be extremely accurate, but are tedious to operate. However, for control purposes, modifications may be made to simplify them and give relative values.

Adsorption of both vapours and solutes may be modified to allow quick control methods to be carried out. If the properties of the powder are established, a size range is chosen so that a change in surface area may be monitored by a particular vapour or solute. The surface area may be determined by reference to results obtained by an absolute method. This method is most useful in the characterisation or control of finely divided surface catalysts.

REFERENCES—

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4. Harkins, W. D., and Jura, G., *Ibid.*, 1944, **66**, 1366.
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6. Wooten, L. A., and Brown, C., *Ibid.*, 1943, **65**, 113.
7. Emmett, P. H., and Brunauer, S., *Ibid.*, 1937, **59**, 1553.

Dynamic

(**71**) DYNAMIC METHOD—

This method is essentially a gas-chromatographic technique with the sample powder in place of the normal chromatographic column. A mixture of helium and nitrogen is passed through the sample and the concentration of nitrogen in the exit gas is measured by thermal-conductivity or gas-density methods, and plotted on a recording potentiometer. The change in nitrogen content of the exit gas when the sample is cooled in liquid nitrogen gives a measure of the quantity of nitrogen adsorbed on the sample surface. The adsorption measurement is repeated, with three or four different concentrations of nitrogen in the helium, and the surface area of the sample is calculated from the BET equation (*i.e.*, $\epsilon = N\sigma$).

REFERENCE

Nelsen, F. M., and Eggertsen, F. T., *Anal. Chem.*, 1958, **30**, 1387.

Ellis, J. S., Forrest, C. W., and Howe, D. D., U.K. Atomic Energy Commission DEG Report 229 (CA), H.M. Stationery Office, 1960.

Daeschner, H. W., and Strass, F. H., *Anal. Chem.*, 1962, **34**, 1150.

B.III. ABSORPTION OF RADIATION

(72) LIGHT BEAM TECHNIQUE—

If a suspension of particles is irradiated by light, the particles of size 2 μ and upwards will interrupt the beam in such a manner that the absorption is related to the projected area (see method (**74**)). For particle sizes less than 0·01 μ, the absorption is related to the volume of the particle. For the intermediate range of sizes (between 0·01 and 2 μ), an extremely complicated relationship is found. It is possible, therefore, to derive surface area of a uniform suspension by measurement of optical density, provided that particles in the size range 0·01 to 2 μ are not present in large quantity.

(73) PENETRATING RADIATION TECHNIQUE—

If penetrating radiation (see "Radiometric Techniques," p. 168) is used, the range of particle sizes in which absorption does not bear a simple relationship to surface area alters in accordance with the wavelength of the radiation used. Outside this range, the absorption depends on the mass of material (see method (**15**)).

B.IV. OPTICAL MICROSCOPE MEASUREMENT WITH PARTICLES IN RANDOM ORIENTATION

(**74**) According to the theorem of Cauchy, particle surface area is related to projected area, in accordance with—

$$S = \frac{4\Sigma A}{n}$$

S approaches absolute surface as n increases, provided that there are no re-entrant surfaces and there is negligible sub-microscopic roughness.

ΣA is the sum of the areas of projected images of n convex particles in radom orientation (a condition not usually obtained on a microscope slide).

It follows that if a truly random dispersion on a slide can be achieved, the surface area of the particles is four times the mean projected area.

A procedure for random dispersion of particles and for measuring the projected areas is described by Pidgeon and Dodd.

REFERENCES—

Cauchy, A., *Compt. Rend.*, 1841, **13**, 1060.
Pidgeon, F. D., and Dodd, C. G., *Anal. Chem.*, 1954, **26**, 1823.

The Furildioximes

Part I. The Structure of the Isomeric Furildioximes

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The so-called α-furildioxime prepared by the usual procedures is shown to be an approximately 1 + 1 mixture of α and γ isomers. When heated under reflux with n-pentanol, the γ isomer is converted to α and β isomers, and the mixture is converted in part to the β isomer. Pure α-furildioxime melts at 192° to 193° C, whereas the γ and β compounds melt at 182° to 183° C and about 150° to 152° C, respectively. All three isomers are shown to form nickel complexes.

FURILDIOXIME has been extensively used as a gravimetric and colorimetric reagent for nickel,[1,2,3,4] palladium[5,6] and platinum[6] for many years, and more recently it has found favour as a colorimetric reagent for rhenium.[7]

The substance employed by all the workers cited has been called α-furildioxime and, in the absence of any information to the contrary, it seems likely that most samples were prepared by the oximation of furil with hydroxyammonium chloride and then aqueous recrystallisation by MacNair's method.[8] This procedure is commonly quoted in the text-books[9,10,11] and gives a product melting between 160° and 168° C, the higher figure being widely accepted as the melting-point of the pure α isomer. Subsequently, there has been confusion in the literature about the melting-points to be assigned to the several possible configurations.[12,13]

We found that repeated recrystallisation from water produced a substance that, after it had been dried, melted at 166° to 168° C; however, assay by treating with an excess of nickel solution and then weighing the precipitate gave an apparent purity of only about 55 per cent. (40 to 50 per cent. on material recrystallised only once). In contrast, by repeatedly recrystallising the crude reaction product from ethanol, we were able to obtain a specimen melting sharply at 192° to 193° C that gave, when assayed by the excess of nickel method, a purity of approximately 100 per cent.

These results led us to suppose that the initial reaction product was a mixture of isomeric furildioximes in which the α (anti-) compound was the minor constituent and that the melting-point most commonly attributed to this isomer was incorrect. Shinra and Ishikawa[12] reached a somewhat similar general conclusion, but the configurations they assigned to the three isomers did not correspond with our own tentative findings. Further experiments were therefore conducted to test and amplify our own views, and the results are given below—

1. The "mixture" melting at 167° C (ex-water) was separated by precipitating the red nickel complex with excess of slightly alkaline nickel solution and then evaporating the filtrate to small bulk, when crystals of a second (γ) isomer separated. This compound was recrystallised from water, and the dried product was found to melt at 184° C and to contain 12·8 per cent. of nitrogen. ($C_{10}H_8O_4N_2$ requires 12·7 per cent. of nitrogen.)
2. A melting-point diagram for the system α - γ isomers was obtained from synthetic mixtures derived from the two procedures outlined above. This gave a eutectic point at about 164° C, corresponding to a mixture containing about 55 per cent. of the α isomer.
3. Ultraviolet and infrared spectroscopic examination showed the differences and similarities listed below—

	α	γ
Melting point of isomer, °C	193	183
$\lambda_{max.}$, mμ	270 to 274	272 to 278
ε	27,000	27,000
Absorption attributed to C : N stretching vibrations	1630, 1580 cm^{-1}	1630, 1560 cm^{-1}
Absorption attributed to OH stretching vibrations	3210 cm^{-1}	3275 cm^{-1}

NOTE—Infrared spectra were produced initially with Nujol mulls in a Perkin Elmer Infracord spectrophotometer, but the absorptions quoted throughout this report are the similar but more precise values kindly determined for us by Dr. J. E. Page, with a Perkin Elmer model 21 spectrophotometer (personal communication from Glaxo Research Ltd.). All ultraviolet spectra were obtained with alcoholic or aqueous solutions and a Beckman DU spectrophotometer.

Attempts were then made to produce a sample of β (syn-) compound by heating the so-called α and γ isomers under reflux in n-pentanol for 7 hours. Treatment of pure γ isomer in this way gave a product that consisted largely of the α compound. The mixed α - γ product gave a treacly mass when the pentanol was removed, and this was dissolved in ether and chromatographed twice on silica gel (M.F.C. grade), with ether as eluent. Five fractions were collected, and, after the solvent had been evaporated, the residues were assayed with excess of nickel. The solid obtained from fractions 3 and 4 gave a brown alcohol-soluble nickel complex, which on weighing indicated an apparent purity of 90 per cent., calculated as a 1 + 1 compound of furildioxime and nickel. Other characteristics of this product were—

Melting point, °C	150 to 152
Nitrogen content, %	11·4 ($C_{10}H_8O_4N_2$ requires 12·7)
$\lambda_{max.}$, mμ	272 to 276 ($\epsilon = 27{,}000$)
Absorption attributed to C : N stretching vibrations	1635, 1565 cm^{-1}
Absorption attributed to OH stretching vibrations	3200 to 3260 cm^{-1}

TABLE I

DIFFERENCES IN THE NICKEL COMPLEXES OF THE THREE ISOMERS

Isomer	Colour of complex	Conditions for precipitation of complex	Solubility	$\lambda_{max.}$, mμ	ϵ	Nickel content,* %
α	Red	Nearly neutral	Insoluble in ethanol	290 to 292	52,000	11·8
β	Brown	Nearly neutral	Soluble in ethanol	275 to 287	36,000	24·8
γ	Green	Above pH 8	Slightly soluble in ethanol	274 to 282	37,700	22·6

* A ratio of furildioxime to nickel of 2 to 1 corresponds to a nickel content of 11·8 per cent. and a ratio of 1 to 1 corresponds to a nickel content of 21·3 per cent.

The hydroxyl stretching frequencies shown by the parent dioximes suggest that a higher degree of hydrogen bonding exists in the α isomer than in the γ isomer, and this is consistent with their respective anti- and amphi- configurations. The intermediate position of the β isomer suggests a moderate degree of intramolecular hydrogen bonding that might be expected with the syn-configuration. Such small differences as were observed with the C : N absorptions are also consistent with the previously reported results for α and β monoximes.[14]

From the evidence offered there seems good reason to suppose that the three compounds we have referred to as α, β and γ isomers (melting-points 193°, 152° and 183° C) are, respectively, the anti-, syn- and amphi-stereoisomers of furildioxime. All three form nickel complexes, that formed with the β isomer being least expected in view of the work of Meisenheimer,[15] but it is suggested that its structure may closely resemble that reported for the palladium - β-benzildioxime complex.[16] The ultraviolet spectra of the β and γ complexes (tabulated above) suggest closely similar structures. Other nickel - γ dioxime complexes have been reported and shown to be 1 + 1 compounds.[15,17,18]

Nickel - α-furildioximate is stoicheiometric, and we found its ultraviolet absorption spectrum to be similar to those reported for nickel dimethylglyoximate and nickel - α-furildioximate.[19] However, unlike nickel dimethylglyoximate, it shows no infrared absorption around 1775 cm^{-1}. Absorption in this region is considered[20] to be due to hydroxyl stretching vibrations.

We thank the Directors of Hopkin & Williams Ltd. for permission to publish this paper.

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The Furildioximes

Part II. The Analytical Behaviour of Purified α-Furildioxime, with Particular Reference to the Determination of Rhenium in the Presence of Molybdenum

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Pure α-furildioxime is shown to behave satisfactorily in the colorimetric determination of rhenium, and a new method is developed for its determination in the presence of 500 times as much molybdenum.

After the work described in Part I of this series had been carried out, it seemed desirable to ascertain whether or not pure α-furildioxime would exhibit any unexpected properties in typical analytical procedures that had previously involved use of a mixture consisting predominantly of the γ isomer.

Since the α isomer had been successfully assayed by precipitation of its nickel complex, this aspect was not pursued further, but it was decided to test the reagent in the procedure described by Meloche, Martin and Webb for the colorimetric determination of rhenium.[1] Accordingly, the procedure applicable in the absence of interfering ions was first carried out, and this yielded results identical with those previously reported. The molar extinction of the rhenium complex, calculated from our experiments, agreed with that stated by Meloche and co-workers to within 0·5 per cent. On the assumption that previous workers had used a reagent containing rather less than 50 per cent. of active isomer, it was thought worth while to repeat these experiments with half the previous amount of furildioxime, and, as expected, this was found to be adequate. This fact is particularly significant, because Meloche and co-workers had recognised that a large excess of reagent was essential for maximum colour development and had specified the least amount consistent with this objective.

Although molybdenum does not form a coloured complex with furildioxime it interferes severely with the determination of rhenium, and Meloche, Martin and Webb utilised a preliminary extraction of the xanthate - molybdenum complex into chloroform. In this method the xanthate is first added while the test solution is at pH 9 to 11, and extraction is then carried out in the presence of a relatively high concentration of hydrochloric acid. The aqueous layer is washed twice with chloroform and then treated with a slight excess of 0·3 N potassium permanganate (about 10 drops, according to directions given by Meloche and co-workers) to oxidise any remaining xanthate or its decomposition products. This is necessary to prevent the subsequent colour development being hindered on the addition of stannous chloride and reagent solutions.

We found that the aqueous layer was extremely turbid and required more than 50 ml of 0·1 N potassium permanganate to complete the oxidation. This made it impossible to restrict the final solution to the recommended volume, and, even when due allowance had been made for this, colour development was either completely inhibited or indefinitely delayed. Purification of the potassium ethyl xanthate did not improve colour development to any extent, and various modifications of the conditions of extraction (reduced acidity, for example) failed to prevent considerable decomposition of the xanthate; however, it should be recorded that Kassner employed a similar separation as a preliminary to the determination of rhenium with 4-methylnioxime.[2]

Various other separations were considered, and one or two were tried experimentally; the most promising was the extraction of the molybdenum into chloroform by means of 8-hydroxyquinoline (oxine). Separation of molybdenum from rhenium as a gravimetric operation with oxine was first reported by Geilmann and Weibk,[3] and then by Michailova, Posner and Archipova.[4] Later, Jasim, Magee and Wilson developed a micro-gravimetric separation based on the same principle,[5] and Gentry and Sherrington have reported that the molybdate - oxine complex can be extracted into chloroform in acidic solution.[6] These observations provided the basis for the separation we ultimately developed.

Preliminary separation of the oxine - molybdate complex from a slightly acidic solution buffered with ammonium acetate, with subsequent washing of the aqueous phase with chloroform, was found to yield a solution that failed to develop the full rhenium - furildioxime colour within a reasonable time, and this was attributed to the retention of traces of reduced molybdenum by the aqueous layer. This conclusion was supported by the fact that further treatment with oxine in chloroform did not effect any improvement. Moreover, the presence of sexavalent molybdenum was known to result in the production of a blue colour when stannous chloride was added later, and this did not normally occur. The effect was presumably due to slight reduction of the molybdate by the oxine itself or by traces of impurities. It was therefore decided to oxidise any molybdenum compounds to molybdate with permanganate after the first extraction with oxine, so that a second extraction with oxine in chloroform would be effective. Our expectation was realised in practice, and a satisfactory procedure was developed.

METHOD

REAGENTS—

8-Hydroxyquinoline (oxine) solution—Dissolve 5 g of analytical-reagent grade 8-hydroxyquinoline in 100 ml of chloroform.

Stannous chloride solution—Dissolve 10 g of analytical-reagent grade stannous chloride in 10 ml of concentrated hydrochloric acid, and dilute to 100 ml with water.

Ammonium acetate solution—Dissolve 15 g of analytical-reagent grade ammonium acetate in 100 ml of water.

α-Furildioxime solution—Dissolve 0·35 g of pure α-furildioxime in 200 ml of acetone. (Meloche *et al.* used 0·7 g in 200 ml and reported that the solution turned yellow within 1 day. We observed no instability with a solution of the pure α isomer.)

Standard rhenium solution—Dissolve 1·553 g of potassium perrhenate in 1 litre of water, and dilute 10-ml portions to 100 ml, as required.

$$1 \text{ ml} \equiv 100\ \mu\text{g of rhenium.}$$

Standard molybdate solution—Dissolve 18 g of analytical-reagent grade ammonium molybdate in 1 litre of water.

$$1 \text{ ml} \equiv 10\ \mu\text{g of molybdenum.}$$

(This solution was prepared for use during the development of the method. It is not required during routine determinations.)

PROCEDURE—

The sample solution should contain not more than 600 μg of rhenium and not more than 50 mg of molybdenum per 10 ml, and both should be in the fully oxidised state, as perrhenate and molybdate, respectively. Transfer 10 ml of neutral sample solution to a 100-ml separating funnel, add 3 drops of 5 N sulphuric acid and 15 ml of ammonium acetate solution, and dilute to 45 ml with water. Mix, add 50 ml of oxine solution, and shake until the aqueous layer becomes clear. Reject the chloroform layer, and wash the aqueous phase with 15 ml of chloroform. Reject the washings, add 0·1 N potassium permanganate, dropwise, to the aqueous solution until a slight permanent pink colour is established, and then re-extract with a further 25 ml of oxine solution. Allow the two layers to separate, wash the aqueous layer with two successive 15-ml portions of chloroform, and filter it through a Whatman No. 41 filter-paper to remove any precipitated manganese dioxide. Collect the filtrate in a 100-ml calibrated flask, add 14 ml of 5 N hydrochloric acid, 26 ml of α-furildioxime solution and 10 ml of stannous chloride solution, and dilute to exactly 100 ml with water. Shake, set the solution aside for 1 hour, and measure the optical density at 532 mμ in a 1-cm cell.

RESULTS

In practice, we found that calibration curves constructed by treating aliquots of standard perrhenate solution alone (100 to 600 μg of rhenium), exactly as above, were almost identical with curves derived from similar amounts of perrhenate in the presence of 50 mg of molybdenum, as molybdate. The two nearly congruent graphs were rectilinear and passed through the origin. Our results for the experiments, in which we used the most adverse ratios of molybdenum to rhenium (*viz.*, rhenium was determined in the presence of 50 mg of molybdenum), were—

Rhenium added, µg	100	200	300	400	500	600
Rhenium found, µg	104	204	291	393	491	605

Theoretically, there seems to be no upper limit to the ratio of molybdenum to rhenium that would prevent successful separation, but, because of the relatively large amounts of oxine solution and of chloroform required, the practical limit is set at about 500 to 1, by coincidence the same as that claimed by Meloche, Martin and Webb for their xanthate separation. Up to 100 mg of molybdenum were successfully separated from 100 µg of rhenium in isolated experiments (*i.e.*, not part of a series), but the procedures were tedious.

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An Automatic Digital Thermometric Titrator*

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An apparatus is described for carrying out volumetric analysis wherein the cessation of heat change indicates the equivalence-point. Titrant is added at constant rate to the titrand so that the voltage output of a thermistor temperature transducer varies linearly with time up to the equivalence-point. The voltage change is electronically differentiated to give a square wave of time period equal to the titre. A timing counter is automatically started at the origin of this square wave and stopped when it begins to decline. The titre is thus given in a digital form. The titrator will operate with titrations giving rates of temperature change greater than 0·01° C per second, and results are given for several different titrations.

The fact that volumetric analysis can be performed by measuring the heat liberated or absorbed during a chemical reaction has been realised for some four decades. For example, when 1 ml of M sodium hydroxide is added to 30 ml of M/30 hydrochloric acid the temperature rise is about 0·4° C. Similarly, if M tetrasodium ethylenediaminetetra-acetate and M/30 magnesium chloride are substituted for alkali and acid under similar conditions the temperature falls by about 0·08° C. The energy changes occurring in such reactions are stoicheiometric, that is to say, they are proportional to the amount of substance formed, and so might be used as a measure of this amount.

In practice it is unnecessary to measure the absolute heat of reaction, and in thermometric titration the cessation of heat liberation or absorption is taken as the indication of the equivalence-point. Dutoit and Grobet,[1] who used a Beckman thermometer and a Dewar flask, plotted temperature change against the volume of titrant added and thus observed a change in slope at the equivalence-point of the reaction. This method of recording temperature is tedious, and in the last decade research workers have used thermistors, resistors with high temperature coefficients of resistance, to detect the extremely small temperature changes encountered in these titrations. Several workers in America[2] have suggested that this principle of measuring heat changes or thermometric titration could be of great value, since the equivalence-point of a reaction followed in this way would not be obscured by other effects such as colour of solution or poisoning of electrodes. If suitable precautions were taken, side effects owing to dilution or stirring could be avoided, since these would be included in the standardisation process.

Jordan and Alleman[3] used a syringe burette driven by a synchronous motor that delivered titrant at a constant rate up to about 0·6 ml per minute. The reaction vessel was thermally insulated by being contained in a Dewar flask, and stirring was by means of a 600 r.p.m. glass stirrer. The molar concentration of the titrant was usually about 100 times that of the titrand in order to minimise volume changes and the effect of slight temperature differences. Under these conditions a difference of ±0·3° C could be tolerated. Temperature changes were followed on a recording millivoltmeter, fed from a bridge circuit incorporating a thermistor, at a chart speed of 1 inch per minute. The plot obtained showed a temperature ordinate and a titre (in millilitres or minutes) abscissa. The straight-line portions of the curve were extrapolated to give the initial and equivalence-points, and the distance between these points was measured along the time axis of the chart to give the titre. This method of evaluating the titre is not entirely satisfactory, especially for routine operation, since it is tedious and introduces error. If this equivalence-point could be detected automatically, then the burette could be stopped at this point and the volume of titrant delivered measured. Alternatively, the time between the initial and equivalence-points might be automatically measured and given in a digital form, since this time is proportional to the volume of titrant delivered.

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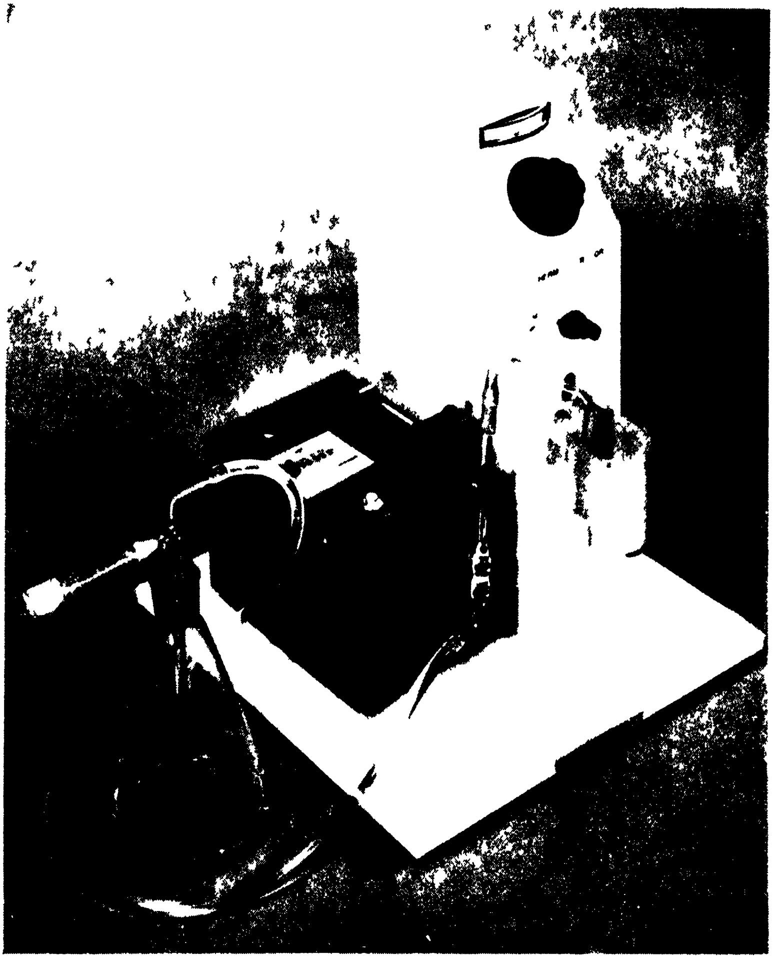

Fig. 1 Digital thermometric titrator

The apparatus described here does, in fact, perform these operations. The main requirements of such a titrator are not only the ability to detect small temperature changes, but also the ability to discriminate between low orders in rate of temperature change (0·01° C per second) necessary for equivalence-point detection. As previously stated, thermistors have sufficient sensitivity, their characteristics satisfy the first requirement, and, consequently, they are utilised in the digital thermometric titrator. The other requirement, which is more difficult to attain, was satisfied by differentiation of the temperature - time curve.

Zenchelsky and Segatto[4] in their paper on derivative thermometric titrations pointed out that an increase in the rate of addition of titrant would be advantageous, since this would increase the rate of temperature change and thus permit the first or second differential of the temperature - time curve to be used to obtain sharper equivalence-point discrimination. They suggested, however, that a limit to such an increase in rate of addition of titrant would be set by problems of heat transfer, and gave 2 ml per minute as their estimate of the limit as applied to a volume of about 25 ml of titrand. During some earlier work in this laboratory, in which high-speed mixing was required, it had been found that a vibrator driving a Perspex paddle drilled obliquely with a number of holes was much more effective and convenient than other methods tried, and consequently was adapted for use in thermometric titrimetry. It has, in fact, been found that the efficiency of stirring allows use of a titrant addition rate of up to 10 ml per minute, with a reduction in both titration time and the effect of small differences between temperatures of titrant and titrand. In the apparatus described here a delivery rate of 7 ml per minute has been utilised.

GENERAL DESCRIPTION AND MODE OF OPERATION

Fig. 1 shows the external appearance of the titrator, its general layout and the control positions. It consists of a constant-delivery-rate burette, the black component, and a white tower that acts as a stand for the titration vessel and also accommodates the transistor circuitry necessary for the operation of the titrator. Titrant, which is drawn from a reservoir into a glass syringe, is delivered into the titrand, held in the titration vessel, when the syringe-piston is driven into its barrel. A timing counter is automatically started when the temperature of the titrand begins to change and is stopped at the equivalence-point of the titration. The titre can then be read from the counter in a digital form.

The mode of operation of the titrator may be more easily understood by reference to Fig. 2. When the titration is begun the temperature of the titrand begins to change at a constant rate with respect to time. The thermistor transducer converts this temperature change into an electrical signal, which takes the form of a linear decrease in voltage, for an exothermic titration, up to the equivalence-point. A filter circuit smooths out stirring noise, and a resistance - capacitance network differentiates the signal to give a square-wave voltage of amplitude proportional to the rate of temperature change and of time period equal to the duration of this change. This voltage is then amplified about 1000 times by a conventional

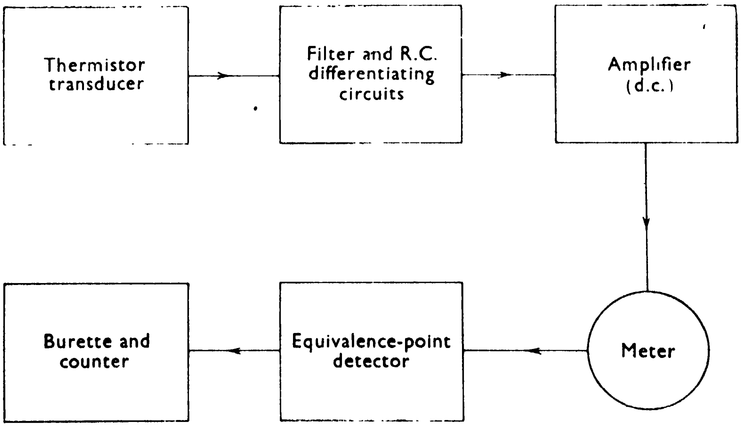
Thermistor transducer
Filter and R.C. differentiating circuits
Amplifier (d.c.)
Meter
Equivalence-point detector
Burette and counter

Fig. 2. Basic schematic diagram

transistor long-tailed pair amplifier to give an output of workable size. The equivalence-point detector consists of a transistor relay circuit that switches on at the change of voltage, *i.e.*, as soon as the thermistor detects a change in temperature, and switches off when the temperature stops changing. Thus the detector relay automatically starts and stops the timing counter, in the burette, at the appropriate points. When the timing counter stops the burette ceases to deliver titrant and automatically refills.

CONSTRUCTIONAL DETAILS

BURETTE—

The constant-delivery-rate burette, which incorporates the timing counter and utilises a motor-driven interchangeable glass syringe, is housed in the black Perspex box (see Fig. 1) and can be easily removed from the white Perspex base, on which it is located by two spigots. The motive power is supplied by a synchronous motor having a constant speed of 3000 r.p.m. and the drive is transmitted to a micrometer screw having 40 turns per inch via a 30:1 reduction worm gear, thus giving a screw speed of 100 r.p.m. The micrometer screw is supported at its ends in bearings, and a nut is allowed to travel along the length of the screw when the latter rotates. A vertical pillar in contact with the end of the syringe piston is connected to this nut. Consequently, when the screw rotates the pillar moves in a longitudinal direction, pushing the piston into its barrel. Microswitches are suitably placed near the ends of the micrometer screw to limit the travel of the pillar to 1 inch and prevent damage. In order to allow withdrawal of the syringe piston, a stainless-steel clip connects the end of the piston to the pillar. A small gap ($\frac{1}{16}$ inch) between the two allows the pillar to attain maximum velocity before it strikes the end of the syringe piston.

The 5-ml interchangeable glass syringe is modified at its delivery end as described below (see Fig. 1). The Luer tip is removed, the hole is sealed, and two small holes are formed on the periphery of the barrel, close to the tip end, one above the other and on opposite sides. Two glass non-return valves, fitted with mercury-weighted ground-glass inserts, are then fused at right angles to the barrel, use being made of the holes previously blown, and are arranged in a vertical axis, one above the other, with an upward flow path through each when the syringe barrel is horizontal. The delivery arm, which directs titrant into the reaction vessel, has a short length ($\frac{1}{8}$ inch) of 0·2-mm capillary at its end to act as a jet to ensure a rapid delivery velocity of 370 cm per second, at a rate of 7 ml per minute, into the titrand. The jet is arranged to be in the "nine-o-clock" position when the titration vessel is viewed from the front. The lower valve is connected to a sintered-glass filter and thence to the titrant reservoir.

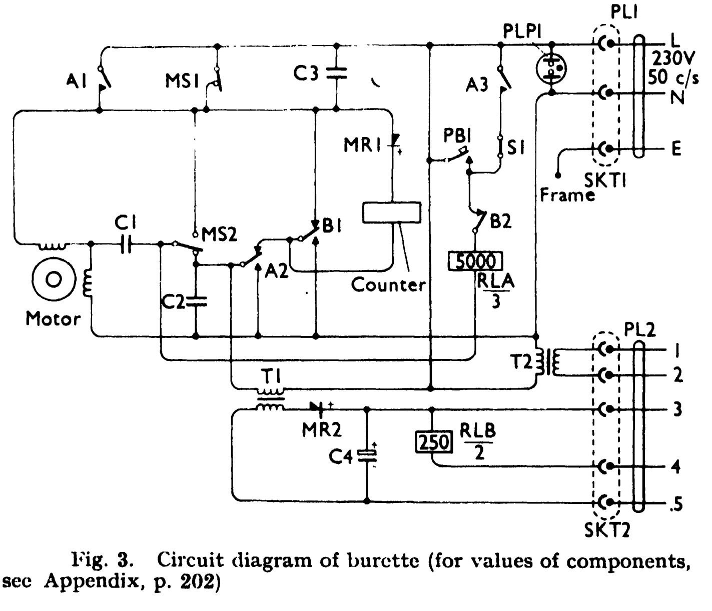

Fig. 3. Circuit diagram of burette (for values of components, see Appendix, p. 202)

When the piston is driven into the barrel, internal pressure causes the lower valve to close and the upper one to open, allowing titrant to enter the titration vessel. As the piston is withdrawn from the barrel, reduced pressure in the latter causes the upper valve to close and the lower one to open, allowing fresh titrant to enter in preparation for the next delivery.

EXPLANATION OF BURETTE CIRCUIT (FIG. 3)

All relay contacts and switches are shown in their normal (off) positions. It is important that relay B should have a smaller time constant than that of relay A, so that at any instant relay B may be energised before the contacts of relay A start to change over. The motor is wired so that relay A when energised effects titrant delivery. When switch S_1 (situated at the rear of the burette) is in the position shown, automatic delivery is obtained in the manner described below. A momentary depression of the start button PB_1 energises relay A via contacts B_2, the motor-start capacitor, C_1, and the motor coil, and it is held on by contacts A_3 and S_1. Contacts A_2 connect C_1 across one motor coil for forward motion (delivery), and A_1, by-passing the stop microswitch, MS_1, which is held open by the pillar when in its stop position, supplies mains current to the motor. The syringe piston will now be driven into the barrel, effecting delivery until the burette pillar hits the reversing microswitch, MS_2. C_1 is then connected across the other motor coil for reversal and refill, and relay A is de-energised. Contacts A_1 open, but the motor does not stop since stop microswitch MS_1 is closed; A_3 open and A_2 revert to their normal positions preventing re-energisation of relay A if the start button is depressed again. The piston is now automatically withdrawn from the barrel effecting refill and, when the burette pillar strikes stop microswitch MS_1, the latter opens and the motor stops.

Since there is a time delay between depression of the start button and the instant at which the thermistor detects the heat change in the titration vessel, the timing counter is remotely started by an impulse transmitted to the burette at points 4 and 5 of socket SKT_2. Thus, when the burette is started and points 4 and 5 are automatically shorted in the tower by a temperature change, relay B is energised. Contacts B_1 change over, and the counter is connected to the neutral line causing it to register time at a rate of 50 counts per second. Contacts B_2 open and de-energise relay A, causing contacts A_1 and A_3 to open and A_2 to change over, thus ensuring that C_1 remains connected to the neutral line and that delivery continues uninterrupted. At the equivalence-point of the titration being performed, points 4 and 5 of SKT_2 are no longer shorted, and relay B is de-energised; this in turn stops the timing counter, reverses the motor and effects refill. While the motor is reversing, the primary connections of transformer T_1 are both at the same potential, and consequently relay B cannot be re-energised if the points 4 and 5 of SKT_2 are again shorted.

Transformer T_2 supplies 6·3 volts at 1·5 amps to the tower via points 1 and 2, and operates the vibrator unit used to effect stirring.

The safety factors listed below have been taken into account—

(1) The burette cannot be started if points 4 and 5 are shorted.

(2) The counter will not operate until the burette is delivering titrant and points 4 and 5 are shorted.

(3) The counter and delivery cannot be restarted, after points 4 and 5 have opened at the equivalence-point, until the burette has refilled and stopped.

(4) If the start button is still depressed when the piston reaches the end of its forward travel, the pillar pushing the syringe piston will automatically reverse and effect refill.

(5) If the equivalence point is not reached at the end of the forward travel, refill should occur since the thermistor will normally detect a break in addition of titrant and cause points 4 and 5 to open. In practice, a count greater than 22 seconds should be suspect, and the titration repeated with a smaller concentration of the titrand.

It should be noted that any proprietary constant-delivery-rate burette (such as the Metrohm or Meniscomatic) may be used in the apparatus described. Manual control of delivery is acceptable, since it is not essential for delivery to cease at the equivalence-point of the titration. Nevertheless, a timing counter, which can be remotely controlled, is required for determining the duration of the titration. Such a counter should be connected to pins 4 and 5 of PL_3 (see Fig. 4) via a suitable relay circuit, and 6 volts d.c. should be applied to pins 5 and 3 of PL_3 for operation of the pilot lamp, ILP_1.

EXPLANATION OF TOWER (FIG. 4)

STIRRER—

The paddle stirrer is driven by a vibration generator similar to that in a loudspeaker unit. The vibrator, which has a resistance of 3 ohms, is supplied with 50 c/s a.c. current from points 1 and 2 at a voltage of 6·3 volts, and the amplitude of vibration is controlled by the 10-ohm variable resistor, RV_6, situated low on the right-hand side panel of the tower. RV_6 is modified by taking off the last few turns of resistance wire so that, as this resistance is increased towards the maximum value, to reduce the stirring rate, the circuit is broken at this value.

The moving armature of the vibration generator is connected to a horizontal arm, 5 inches long and made of clear Perspex tube $\frac{3}{8}$ inch outside diameter and $\frac{1}{4}$ inch inside diameter, by an aluminium clamp internally lined with sponge-rubber. One end of this arm is joined to a Tufnol mounting containing a ball-race that acts as a pivot. The other end of the arm is mitred to a vertical arm made of the same material and of length $1\frac{1}{2}$ inches. The horizontal arm passes through the slotted front panel of the tower. The small vertical arm acts as a holder for the thermistor, which is fitted with a short rubber sleeve so that the thermistor will remain attached to this arm. The thermistor leads are soldered (this is most important since two types of sockets have proved most troublesome in practice) to a length of coaxial cable that runs inside the horizontal arm and emerges at the pivot end. This arrangement ensures that there can be no corrosion of the thermistor leads, which could cause considerable electronic noise.

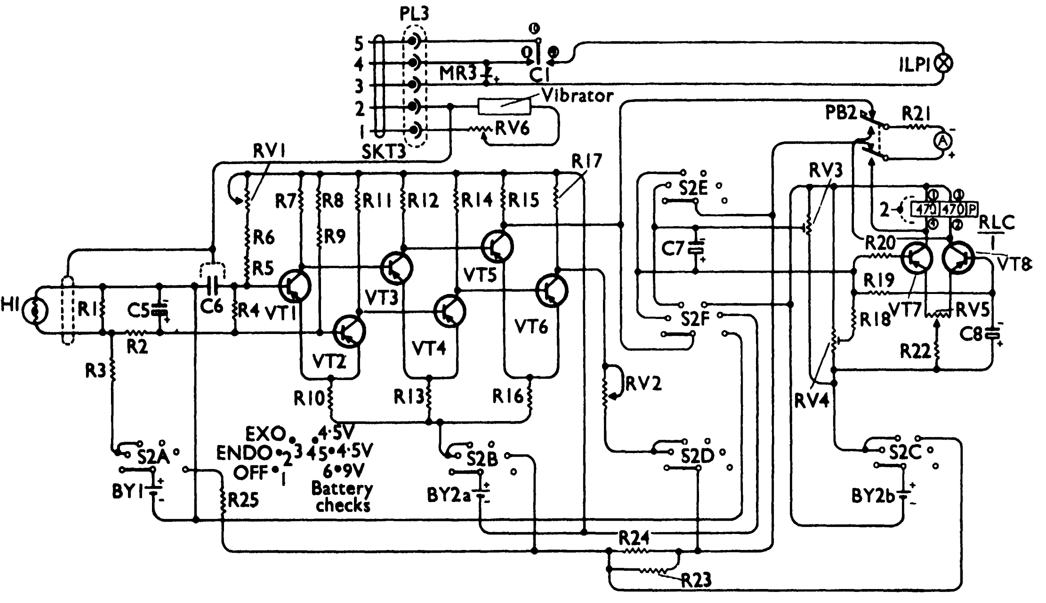

Fig. 4. Circuit diagram of tower (for values of components, see Appendix, p. 202)

The circular paddle is of clear Perspex, $\frac{1}{8}$-inch × $1\frac{1}{2}$-inch diameter, obliquely drilled with holes, six of $\frac{1}{4}$-inch diameter being arranged in a hexagon around the centre of the wheel and others of $\frac{1}{8}$-inch diameter filling in the outer annulus. The paddle is secured to the end of the thermistor with a small rubber sleeve and positioned so that the temperature-sensitive tip of the thermistor protrudes through it.

TITRATION VESSEL AND HOLDER—

The titration vessels are cylindrical polythene beakers, without lips or protrusions, of 2 inches outside diameter × $2\frac{3}{4}$ inches in length. Polythene has proved most suitable because of its good heat insulation and chemical resistance. These vessels are held in a clear Perspex tube, the inside diameter of which is slightly greater than the outside diameter of the vessels, that is mounted on the front of the tower at a height that allows the vessel to be inserted from below. This holder has two finger slots, one on each side, to facilitate removal of the titration vessel after a titration. A polythene clip, screwed to the top of the holder, holds the vessel securely in a vertical position during a titration.

THERMISTOR TRANSDUCER—

A thermistor, TH_1 (Standard Telephone and Cables Ltd. type F23), having a resistance of about 2000 ohms at 20° C and a temperature coefficient of resistance of 0·04 ohm per ohm per °C is used as the temperature-sensing element, and is incorporated in a simple temperature transducer together with resistance R_1. The effective resistance of the combination varies nearly linearly (to within about 3 per cent.) with the temperature of the thermistor, and during an exothermic titration the voltage across R_1 drops at a rate of about 35 mV per °C.

FILTER AND DIFFERENTIATING CIRCUITS—

The signal from R_1 is smoothed by a conventional filter consisting of R_2 and C_5, which also eliminates 50 c/s mains pick-up. The time constant of 100 milliseconds is sufficient and causes only slight attenuation. The voltage variations occurring across C_5 are electronically differentiated by the R.C. network consisting of C_6, a 40-μF paper-insulated capacitor, and the input resistance of the amplifier, which is about 1000 ohms. The time constant of this differentiating circuit is about 40 milliseconds, a compromise that has proved most suitable in practice. The sharpness of detection of voltage change could be increased by reducing the time constant of the differentiating circuit, but this gain would be associated with an attenuated and noisier differentiated output. Further stages of amplification would be required to raise the signal to the previous level and this would itself give rise to amplifier noise, and so the net effect of achieving sharper equivalence-point detection would be to give much more noise.

TRANSISTOR AMPLIFIER—

The magnitude of the differentiated signal developed across R_4 is increased by the 3-stage long-tailed pair amplifier comprising transistors $VT_{1\ \mathrm{to}\ 6}$. This amplifier has a gain of about 1200, and since direct coupling is used the base resistances of VT_1 and VT_2 must be adjusted to get the other transistors working on the correct parts of their characteristic curves. Resistors R_5 and R_9 facilitate this operation. The voltage between the collectors of VT_5 and VT_6 can be brought to zero by the action of the 10-turn 15,000-ohm potentiometer, RV_1, which is the amplifier balance control and is situated just below the balance meter at the top of the tower.

The amplifier output can be attenuated by the sensitivity control, RV_2, which is situated near the top of the right-hand side panel of the tower. This control, which covers a linear range of 4:1, limits the current into the meter and detector-relay circuit. Control RV_2 is fitted with a pointer that is surrounded by a plastic bezel linearly calibrated 0 to 10. When the pointer shows 0 the effective resistance of RV_2 is 1000 ohms; in the position 10 the effective resistance is zero and the sensitivity a maximum. The meter, which reads 50–0–50 μA and has a resistance of 850 ohms, always gives a deflection to the right during exothermic titrations and to the left for endothermic titrations.

RANGE SWITCH—

This switch, S_2, has six poles and six positions and must be of the break-before-make variety. Poles A, B and C switch on the three batteries in positions 2 and 3, whereas in positions 4, 5 and 6 the battery voltages are individually shown on the meter. The meter needle should register between 38 and 50 μA on the scale, and the batteries must be renewed if necessary. Pole D switches the amplifier out of circuit to facilitate these battery checks. The remaining poles E and F transmit or reverse the output of the amplifier to the detector-relay circuit.

BATTERIES—

The two 4·5-volt batteries, BY_{2a} and BY_{2b}, are in one container, and they and the 9-volt battery, BY_1, are fitted with standard irreversible 2-pin plugs. The batteries are kept in a compartment at the base of the tower.

EQUIVALENCE-POINT DETECTOR—

The detector circuit, which is the heart of the apparatus, consists basically of a sensitive differential relay circuit utilising a dual-coil Carpenter relay with bi-stable contacts. The relay contacts C_1 change state when the voltage across capacitor C_7 (mains hum suppressor) quickly changes by more than 30 mV. This voltage may rise to about 200 mV, and if there is no change in direction of this voltage the contacts C_1 remain in the same state, but they will revert to the other position if the voltage suddenly drops by more than 30 mV from a constant value. Consequently, before the start of a titration, contacts C_1 must be in the position where tags 9 and 10 are shorted and this is indicated by the illumination of signal lamp ILP_1, which shines through the transparent casing of the meter. As a titration proceeds a voltage change suddenly occurs across C_7 that causes contacts C_1 to change over, and this in turn starts the timing counter and extinguishes the signal lamp. The diode, MR_3, acts as a spark quenching element, since the switched load, relay B, of the detector Carpenter relay is inductive. The potentials of the sliding contacts of potentiometers RV_3 and RV_4 are set at −0·9 volt and −0·65 volt, respectively, and the collector potentials of VT_7 and VT_8 should be about −2·2 volts. The bias control, RV_5, situated underneath the press button, PB_2, on the side panel of the tower, allows the collector voltages to be made equal since the difference is shown on the meter when PB_2 is depressed.

SETTING-UP AND OPERATION OF TITRATOR

About 30 ml of titrand are placed in the titration vessel, which is then placed in the holder. The stirring rate is increased from zero until "spitting" of the titrand does not quite begin. The range switch is set to the required setting, and the sensitivity control is set to a suitable value. The amplifier balance control is rotated until the meter reads zero, making sure that the signal lamp that shines through the meter is alight. The bias-check press button is then depressed, and the bias control is rotated for zero meter reading.

The burette counter is re-set to zero, and, when the burette start button is depressed, titrant is added to the titrand, and the meter shows a deflection as the counter automatically starts to register elapsed time. At the equivalence-point the meter deflection starts to return to zero and the counter stops. The stirrer is turned off and the titration vessel is removed; finally, the stirring assembly is washed by immersion in distilled water. The count is noted and compared with that for a standard strength titrand so that the titre may be evaluated.

It should be noted that the detector circuit will respond to fluctuations in intensity of sunlight and, for this reason, the titrator should be shielded whenever necessary.

RESULTS AND CONCLUSIONS

The reproducibility of the results obtained has been evaluated by performing several titrations with M hydrochloric acid as titrant and 30 ml of M/30 sodium hydroxide as titrand. The results of 15 replicates gave a mean count of 8·56 seconds and showed a standard deviation of 0·08 seconds. A second series of 15 replicate determinations containing 30 ml of M/15 sodium hydroxide gave a mean count of 17·16 seconds and the same standard deviation of 0·08 seconds.

The stroke of the syringe piston limits the maximum volume of titrant to about 2·5 ml, which at the rate of 7 ml per minute corresponds to a count of 22 seconds. For good precision the minimum volume of titrant used was limited to 0·5 ml, and thus the titrator was operated with titrand concentrations giving counts (titres) in the range 4·4 to 22 seconds.

In order to evaluate the relationship between the concentration of titrand and the count (titre) indicated by the titrator, series of similar titrations were performed. Each series included titrations of the same titrand at different concentrations with a standard titrant, *e.g.*, 30 ml of M/60, M/40, M/30, M/24 . . . M/12 sodium hydroxide with M hydrochloric acid, giving counts between 4·4 and 22 seconds. Further series of titrations (see Table I) were performed with other titrands and M hydrochloric acid as titrant. Results for a range of titrants and titrands are shown in Tables II to V.

The Ferranti Sirius Computer, suitably programmed, was utilised to evaluate, by the method of least squares, the best straight-line relationship between titrand concentration and count (titre) for each series of titrations. The zero intercept in units of time (seconds) is given in column 6 of the Tables, and its magnitude is influenced by the extent of delayed reaction between reactants or the solubility of the reaction product in the titrand. Even in titrations for which the intercept term is large, the method may still be utilised, since a calibration can be employed. The computer was also used to calculate the standard deviation of the line, *i.e.*, the scatter of the results across the best straight line showing the relationship between concentration and count. The results in column 7 (headed "Sigma") are in units of time (seconds) and give an indication of the thickness of the scatter of points around the

TABLE I

TITRATIONS WITH M HYDROCHLORIC ACID AS TITRANT

Titrand	C_m	Type	Setting	No. of titrations	Intercept, seconds	Sigma, seconds	Sigma, %
$NaOH$	M/60	+	0	10	−0·05	0·09	0·6
$AgNO_3$ ..	M/60	+	0	10	−0·35	0·08	0·5
NH_4OH ..	M/60	+	0	10	−0·19	0·17	1·1
Na_2CO_3 ..	M/60	+	8	10	−0·32	0·07	0·5
$NaHCO_3$ ..	M/60	+	10	7	−0·33	0·05	0·3
$Na_2B_4O_7$ ..	M/60	+	10	6	−0·29	0·12	0·8
Na_3PO_4 ..	M/60	+	5	10	−0·28	0·09	0·6
$Na_2S_2O_3$ ..	M/60	+	10	5	−0·07	0·13	0·8
KI + excess of KIO_3 ..	M/60	+	5	5	−2·22	0·18	1·2
$Na_4(EDTA)$ ..	M/120	+	5	7	−0·75	0·03	0·2
KIO_3 + excess of KI ..	M/360	+	5	6	−0·71	0·03	0·2
NaH_2PO_2 ..		—	(Insufficient heat to give an end-point)				
$NaHSO_3$ ..		—	(Insufficient heat to give an end-point)				

TABLE II

TITRATIONS WITH M TETRASODIUM EDTA AS TITRANT

Titrand	C_m	Type	Setting	No. of titrations	Intercept, seconds	Sigma, seconds	Sigma, %
$Ca(NO_3)_2$ ..	M/60	+	9	8	−0·15	0·15	1·0
$NiSO_4$	M/60	+	8	11	−0·11	0·10	0·6
$CuSO_4$	M/60	+	8	11	+0·02	0·08	0·5
$CdSO_4$	M/60	+	10	11	−0·16	0·07	0·5
$ZnSO_4$	M/60	+	10	5	−0·01	0·06	0·4
$BaCl_2$	M/60	+	10	10	−0·01	0·14	0·9
$AgNO_3$ ..	M/60	+	5	4	−1·15	0·32	2·0
$CoCl_3$	M/60	+	10	4	−0·93	0·05	0·3
$Ce(SO_4)_2$ ..	M/120	+	0	7	−0·27	0·14	0·9
$SnCl_4$	M/120	+	8	10	−0·17	0·09	0·6
$Cr(NO_3)_3$ ..	M/60	—	7	3	+0·56	0·03	0·2
$AlCl_3$	M/60	—	0	8	−0·21	0·12	0·8
$Mg(NO_3)_2$ ..	M/60	—	7	5	−0·02	0·25	1·6
$Be(NO_3)_2$ ..	M/60	—	6	6	+0·03	0·21	1·4
$LiNO_3$		+	(Insufficient heat to operate titrator)				
NH_4NO_3 ..		—	(Insufficient heat to operate titrator)				
$Sr(NO_3)_2$ ..	M/60	+	5	(No results)			
$Hg(NO_3)_2$ ..	M/60	+	0				
$Fe(NO_3)_3$ ..	M/60	+	0				
$Fe(NO_3)_2$ ..	M/60	+	0				
$MnCl_2$	M/60	+	10				

TABLE III

TITRATIONS WITH M SODIUM CARBONATE AS TITRANT

Titrand	C_m	Type	Setting	No. of titrations	Intercept, seconds	Sigma, seconds	Sigma, %
$Pb(NO_3)_2$ ..	M/60	+	10	4	−0·34	0·21	1·4
$AgNO_3$	M/30	+	0	8	+0·15	0·17	1·1
HCl	M/30	+	2	6	0·00	0·08	0·5
$Zn(NO_3)_2$ ..	M/60	—	6	7	−1·48	0·17	1·1
$CuSO_4$	M/60	—	8	6	−3·20	0·13	0·8
$AlCl_3$	M/90	—	6	8	−3·71	0·44	3·0

TABLE IV

TITRATIONS WITH M/4 CERIC SULPHATE IN SULPHURIC ACID AS TITRANT

Titrand	C_m	Type	Setting	No. of titrations	Intercept, seconds	Sigma, seconds	Sigma, %
KI	M/120	+	10	8	−3·08	0·24	1·5
Na_2SO_3.. ..	M/240	+	10	13	−0·54	0·11	0·7
$Na_2S_2O_3$ ..	M/240	+	10	8	−1·33	0·06	0·4
Hydroquinone	M/600	+	5	8	−0·80	0·06	0·4
Metol	M/240	+	5	7	−0·77	0·06	0·4
Phenidone ..	M/600	+	10	11	+0·19	0·27	1·8
Resorcinol ..	M/480	+	8	8	−1·00	0·12	0·8

TABLE V

TITRATIONS WITH 2 M SODIUM THIOSULPHATE AS TITRANT

Titrand	C_m	Type	Setting	No. of titrations	Intercept, seconds	Sigma, seconds	Sigma, %
I_2 in KI	M/60	+	10	8	−0·05	0·24	1·5
$Ce(SO_4)_2$ in H_2SO_4	M/30	+	10	8	+0·82	0·10	0·6
Na_2SO_3 in KI_3 (titration of excess of I_2)* ..	M/1000	+	10	6	+0·02	0·06	0·4
$NaHSO_3$ in KI_3 (titration of excess of I_2)* ..	M/1000	+	10	6	+0·01	0·08	0·5
Hydroquinone in $K_2Cr_2O_7$ + H_2SO_4 (titration of excess of $Cr_2O_7^{2-}$)†	M/4000	+	0	4	−0·13	0·22	1·4

* Maximum concentration M/12.

† Maximum concentration M/50.

best straight line drawn through the points. Column 8 gives the coefficient of variation of the scatter for titrations giving a count of 15 seconds. Column 2 gives the minimum concentration of C_m of titrand that can be used with the standard titrant and the maximum concentration is in all cases 5 C_m, unless specified to the contrary. Column 3 (headed "Type") indicates the type of reaction, + denoting exothermicity and — endothermicity. Column 4 (headed "Setting") gives the values of the sensitivity setting utilised in the series of titrations; column 5 shows the number of titrations in each series. Thus the first line in Table I indicates that sodium hydroxide was titrated against M hydrochloric acid in a concentration range of M/60 to M/12 with the titrator range switch at the position "EXO" and the sensitivity pointer at position 0. The intercept is —0·05 seconds, which means that if 0·05 is subtracted from the count for each of the ten titrations in this series then the straight-line relationship between concentration and count would pass through the origin. The coefficient of variation of the scatter for a count of 15 seconds is ±0·6 per cent.

The concentrations of titrants used were convenient values, but it should be noted that, although an increase would produce more heat due to reaction, this would not always give rise to a greater temperature change, since the heat of dilution might be in the opposite sense.

Other titrants not included in the Tables have been utilised at concentrations ensuring a rate of temperature change of 0·01° C per second or more; for example, argentometric titrations with M/5 silver nitrate as titrant and precipitation titrations with M/5 sodium sulphide.

Appendix

LIST OF COMPONENTS USED IN THE CONSTRUCTION OF THE APPARATUS

(Figs. 3 and 4)

R_1, R_7, R_{11} = 2200-ohm resistors.
R_2, R_{16} = 1000-ohm resistors.
R_3, R_{18} = 3300-ohm resistors.
R_4, R_6, R_8, R_{23}, R_{25} = 100,000-ohm resistors.

R_5, R_9 = Selected values (see text).
R_{10} = 100-ohm resistor.
R_{12}, R_{14} = 220-ohm resistors.
R_{13}, R_{15}, R_{17} = 150-ohm resistors.
R_{19}, R_{20} = 470-ohm resistors.
R_{21} = 10,000-ohm resistor.
R_{22} = 33-ohm resistor.
R_{24} = 1-megohm resistor.
RV_1 = 15,000-ohm 10-turn potentiometer (Colvern Ltd.).
RV_2, RV_3 = 1000-ohm 1-turn potentiometers (Colvern Ltd.).
RV_4 = 2000-ohm 1-turn potentiometer (Colvern Ltd.).
RV_5 = 5-ohm 1-turn potentiometer (Colvern Ltd.).
RV_6 = 10-ohm 1-turn potentiometer (Colvern Ltd.).
TH_1 = Stantel F23 thermistor, 2000-ohms at 20° C.
C_1 = 1·25-μF paper-insulated capacitor, 350-volt working.
C_2, C_3 = 0·1-μF paper-insulated capacitors, 350-volt working.
C_4 = 50-μF electrolytic capacitor, 12-volt working.
C_5, C_7 = 100-μF electrolytic capacitors, 6-volt working.
C_6 = 40-μF paper-insulated capacitor, 150-volt working.
C_8 = 2000-μF electrolytic capacitor, 6-volt working.
VT_1, VT_2, VT_7, VT_8 = Mullard OC202 transistors.
VT_3, VT_4, VT_5, VT_6 = Mullard OCT75 transistors.
RLA = 230-volt 50 c/s coil, 3-pole, C/O 5A (M.T.I. Ltd.).
RLB = 6-volt d.c. coil, 2-pole, C/O 5A (Siemans Halske).
RLC = Carpenter relay, 1-pole, C/O 5C6 (Telephone Mg. Co. Ltd.).
T_1 = 230-volt 50 c/s primary, 8-volt 0·5-amp secondary transformer.
T_2 = 230-volt 50 c/s primary, 6·3-volt 1·5-amp secondary transformer.
MR_1 = Westinghouse 15 RC1-1-16 1 rectifier.
MR_2, MR_3 = Mullard OA10 diodes.
MS_1, MS_2 = Honeywell Brown 11 SM1-T microswitches.
PB_1, PB_2 = Honeywell Brown 2 PB 12 T press buttons.
S_1 = Single-pole switch.
S_2 = 6-pole 6-way rotary switch, break-before-make (Painton Ltd.).
BY_1 = Vidor 6007 9-volt battery.
BY_2 = Vidor 6011 twin 4·5-volt battery.
PLP_1 = Neon indicator lamp.
ILP_1 = Pilot bulb, 6·3-volt 0·115 amp.
PL_1, SKT_1 = 4-way plug and socket (Plessey Ltd.)
$\left.\begin{array}{l}PL_2, SKT_2\\ PL_3, SKT_3\end{array}\right\}$ = 6-way plugs and sockets (Plessey Ltd.)

MISCELLANEOUS COMPONENTS

Counter F43/G3/J46 (Lancashire Dynamo Co. Ltd.).
Worm and worm wheel 30:1 (Muffets Ltd.).
Micrometer screw (Moore and Wright Ltd.).
5-ml syringe (Chance Glass Ltd.).
Synchronous motor FC84/0 (Evershed Vignoles Ltd.).
Vibration generator V47-3 (Goodman Ind. Ltd.).
Meter, 50 0–50 μA, type 220 (Taylor Ltd.).
Weight of burette = 7 lb (3·2 kg).
Weight of tower and base = 14 lb (6·4 kg).

I thank Messrs. A. D. Johnson and D. M. Zeitlin of Kodak Ltd. who processed the results and programmed the computer.

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Quantitative Determination of Glucose and Maltose in Enzyme Reaction Mixtures

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The application of a carbon-column selective-desorption technique is shown to be suitable for determining maltose and glucose in enzyme reaction mixtures. Precipitation of the enzyme protein and removal of the buffer ions is unnecessary for both the column separation and sugar determination by the copper colorimetric method adopted. In enzyme reaction mixtures containing initially 50 mg of maltose, the maltose and glucose concentrations can be determined, when the amount of hydrolysis is within the range 1 to 80 per cent., with a maximum error of about 2·5 per cent.

MANY of the methods available for determining each component in glucose - maltose mixtures are time consuming and of limited accuracy. The accurate selective-desorption of sugars from columns of activated carbon by the method of Patterson and Savage[1] and Patterson and Buchan[2,3] appeared to be suitable, after modification, for the quantitative determination of glucose and maltose in enzyme reaction mixtures. However, the effect of enzyme preparations and buffer ions on the determination was unknown, since Patterson and co-workers did not use enzyme reaction mixtures.

Before determining reducing sugars in biological liquids it is customary to precipitate the proteins. Since the protein content in these enzyme reaction mixtures was small, it was decided to investigate the effect of not precipitating the protein, as such a procedure would considerably accelerate the determination.

METHOD

REAGENTS—

Activated carbon—"Activated charcoal powder for decolorising purposes: washed with acid," obtained from British Drug Houses Ltd., Poole, Dorset, was used.

Kieselguhr—The material purified with acid, obtained from British Drug Houses Ltd., was used.

Somogyi[4] *copper reagent*—Dissolve 28 g of anhydrous disodium hydrogen orthophosphate and 40 g of sodium potassium tartrate in 700 ml of hot water. Add 100 ml of sodium hydroxide and 80 ml of a 10 per cent. solution of copper sulphate, $CuSO_4.5H_2O$. Cool, make up to 1 litre, and then add 180 g of anhydrous sodium sulphate. Store at 25° + 5° C, and filter, if necessary, after 48 hours. This reagent was preferred to a later Somogyi[5] reagent chiefly because the latter formed persistent small bubbles after the addition of the Nelson reagent. These were difficult to remove and they seriously interfered with the colour measurement.

Nelson[6] *chromogenic reagent*—Dissolve 25 g of ammonium molybdate in 450 ml of water, and add 21 ml of concentrated sulphuric acid. Dissolve 3 g of sodium arsenate, $Na_2HAsO_4.7H_2O$, in water, and mix thoroughly with the molybdate - sulphuric acid solution. Make up to 500 ml, and incubate at 37° C for 48 hours. Filter, and store in a dark-brown bottle at room temperature.

Universal buffer mixture—Obtained from Hopkin & Williams Ltd., Chadwell Heath, Essex.

Enzyme preparation—Two freeze-dried preparations were made from the gut of the desert locust, *Schistocerca gregaria* (Forsk). One consisted of foregut and midgut tissue and the other of foregut and midgut contents. The tissue was homogenised and spun in a centrifuge (3000 **g**) for 10 minutes. The contents were shaken with a minimum of water, and then spun in a centrifuge in the same manner as for the tissue. The supernatant liquids were dialysed against distilled water for 48 hours at 2° C, and finally spun in a centrifuge for 45 minutes at high speed (22,000 g) before being shelled. Kjeldahl analysis of the enzyme preparations showed that the tissue preparation contained 11 per cent. of nitrogen and the contents preparation contained 7 per cent. of nitrogen.

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ENZYME REACTION MIXTURE AND PROCEDURE—

Although the total volume of reaction mixture was varied, the proportion of the constituents were generally kept the same. A typical reaction mixture was 1 ml of a 5 per cent. solution of maltose, 2 ml of buffer solution at pH 5·7 and 1 ml of enzyme extract containing 1 mg of enzyme preparation.

The carbon-column assembly was similar to that described by Patterson and Savage.[1] The temperature of the column was maintained at 38° ± 1° C, and the temperature of the eluting liquids in the reservoir at the top of the column was kept within this range by suitable lagging. The suction pressure on the column was arranged so that 100 ml of eluate were collected in about 40 minutes. To avoid overloading the column with sugar, it was necessary to restrict the amount of reaction mixture placed on the column, so that the maximum amount of maltose was 12·5 mg. Equal volumes of eluate were collected for glucose and maltose determinations, the former being eluted first with water and maltose afterwards with 7 per cent. ethanol.

The qualitative separation of sugar mixtures was examined by chromatographic analysis of the eluates after (*a*) a mixture containing 5 mg of glucose and 5 mg of maltose, (*b*) a mixture containing 5 mg of glucose, 5 mg of maltose and 5 mg of isomaltose and (*c*) a solution containing 5 mg of glucose had been passed through the column. The eluates were concentrated by controlled evaporation to 0·2 ml. Appropriate sugar markers and approximately 2·5 µl of the concentrated eluates were placed on the chromatography paper (Whatman No. 1 filter-paper), which was then irrigated by an ascending-solvent technique with a mixture of n-butanol, pyridine and water in the ratio 6 to 4 to 3 parts by volume. The chromatogram was then dried and developed to show the position of the sugars by Trevelyan, Proctor and Harrison's silver method.[7] Table I shows that qualitative separation of glucose and maltose was achieved, but isomaltose was eluted with maltose.

TABLE I

MOVEMENT OF SUGARS PRESENT IN COLUMN ELUATES COMPARED WITH STANDARD MARKERS

	R_G value	Sugar identification
Markers—		
Glucose	1·00	Glucose
Maltose	0·64	Maltose
Isomaltose	0·48	Isomaltose
Eluate concentrates—		
1. Glucose - maltose mixture—		
Water eluate	0·95	Glucose
Ethanol eluate	0·65	Maltose
2. Glucose - maltose - isomaltose mixture—		
Water eluate	0·97	Glucose
Ethanol eluate	0·64, 0·47	Maltose, isomaltose
3. Glucose solution—		
Water eluate	0·94	Glucose
Ethanol eluate	—	—

For the quantitative determination of glucose and maltose in their eluates, the colorimetric method of Somogyi[4] and Nelson[6] was modified. Four millilitres of eluate and 4 ml of Somogyi reagent were heated in a boiling-water bath for 15 minutes (glucose) or 20 minutes (maltose) in a 30-ml tube, the mouth of which was closed by a glass bulb. The tube was then immediately transferred to a cold-water bath for 20 minutes. Nelson's chromogenic reagent was added, the tube was well shaken, and the contents were made up with water to 10 or 25 ml, according to the colour intensity. Maltose eluates were always made up to 25 ml. Eluates containing less than 0·25 mg of glucose were also made up to 10 ml, but eluates containing 0·25 to 0·65 mg of glucose were made up to 25 ml. The optical densities of the coloured solutions were measured after 25 ± 5 minutes with a Unicam SP600 spectrophotometer at 500 mµ. For the determination of sugars in eluates of non-enzymic solutions, which were used for calibration purposes, the spectrophotometer was adjusted so that zero optical-density readings were given by the reagent blank solutions containing 4 ml of water or 7 per cent. ethanol instead of 4 ml of water or ethanol eluate, respectively.

The sugar content of the enzyme reaction mixtures was measured after samples had been immersed in a boiling-water bath for 1 minute to denature the enzyme. Reagent blank solutions for glucose determinations were made up from 4 ml of the aqueous eluate after denatured zero-incubation time samples of enzyme reaction mixture had been placed on the column. This procedure was not possible for the determinations of maltose, for which control reaction mixtures were made up with water instead of maltose solution, and samples were placed on the column. Four millilitres of the ethanol eluate were then used to make up the reagent blank solution. Eluates containing maltose and glucose below the range of the calibration curves were concentrated by controlled evaporation before the Somogyi reagent was added. Eluates containing glucose and maltose above the range of the calibration curves were suitably diluted with water.

CALIBRATION—

The calibration curve for maltose was linear, the maximum amount of maltose being 1·6 mg. Samples containing 1 mg of maltose gave an optical-density reading of 0·639. Two linear calibration curves were also obtained for glucose. When the final volume of coloured solution was made up to 10 ml (0·25 mg of glucose), 0·1 mg of glucose gave an optical-density reading of 0·296, and when the final volume of coloured solution was made up to 25 ml (0·25 to 0·65 mg of glucose), 0·5 mg of glucose gave an optical-density reading of 0·607.

EFFECT OF ENZYME PROTEIN ON THE DETERMINATION OF GLUCOSE—

Addition of enzyme to solutions of glucose increased their reducing power. Fig. 1 shows the effect of adding 1 mg of enzyme preparation (gut contents) to glucose solutions before treatment with Somogyi reagent, and then determining their reducing power.

The change in reducing power is most marked in extremely dilute solutions of glucose when an increase of 7 per cent. was found compared with a 3 per cent. increase in solutions containing 0·2 mg of glucose. For solutions containing 0·2 to 0·6 mg of glucose, the increase in reducing power was less than 3 per cent. This enzyme protein effect, which was found to be approximately proportional to the amount of enzyme added, was about three times as great with the tissue preparations as with the contents preparation. In usual enzyme reaction mixtures, in which the relative amount of enzyme is a quarter of that used for obtaining the results in Fig. 1, the protein effect of the contents preparation was approximately 2 per cent. for extremely small amounts of glucose and less than 1 per cent. for amounts of glucose greater than 0·2 mg. The magnitude of the error is not unreasonable, particularly

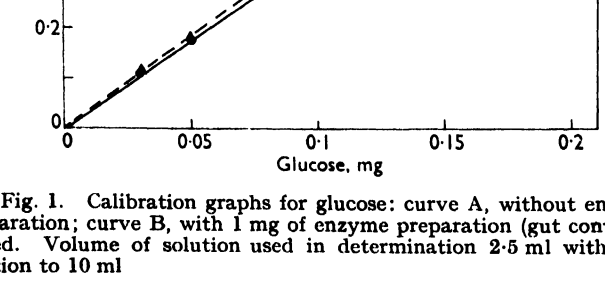

Fig. 1. Calibration graphs for glucose: curve A, without enzyme preparation; curve B, with 1 mg of enzyme preparation (gut contents) added. Volume of solution used in determination 2·5 ml with final dilution to 10 ml

when the disadvantages of the alternative procedure of protein precipitation are considered, *viz.*, time and dilution factors. The enzyme protein was therefore not precipitated from reaction mixtures before analysis.

Recovery of sugars from the carbon column—

Table II shows a typical set of calibration results for the recovery of sugar from pure solutions and mixtures and from solutions containing buffer salts and denatured tissue enzyme preparation. When glucose and maltose solutions were used separately, the amount of sugar recovered was slightly less than that present in the original solution. The analysis of mixtures, however, indicated a further drop in maltose recovery, but an increase in glucose recovery. The results from many determinations indicate that there was at least 98 per cent. recovery of maltose and glucose when used separately, and that a recovery of 102 per cent. was obtained for glucose when used in a mixture with maltose, but the recovery of maltose (97·5 per cent.) was slightly less. These recoveries can be accounted for to a certain extent by the fact that, although the glucose preparation was chromatographically pure, the maltose preparation exhibited a trace of glucose on the chromatogram. The addition of buffer and enzyme preparation to glucose - maltose mixtures affected the recovery of sugar only to a small extent. When reaction mixtures containing ten times the usual amount of enzyme preparation were analysed, the average glucose gain was 2 per cent. and the average maltose loss 3 per cent. The presence of 0·25 mg per ml of denatured enzyme preparation and buffer ions in glucose - maltose mixtures therefore has a negligible effect on the recovery of the sugar.

Determination of sugar in enzyme reaction mixtures—

The hydrolysis of maltose can be followed quantitatively by measuring the decreasing maltose concentration and/or the increasing glucose concentration. Fig. 2 shows that the progress curves in relation to maltose disappearance and glucose appearance follow a corresponding pattern. However, the total sugar recovered decreases progressively with time. This was due to the formation of transfer sugars, the presence of which were established by paper-chromatographic analysis.[8] Transfer disaccharides, trisaccharides and higher oligosaccharides were formed (full details of which it is hoped will be published shortly), and these were presumably not removed from the column by elution with water and 7 per cent. ethanol.

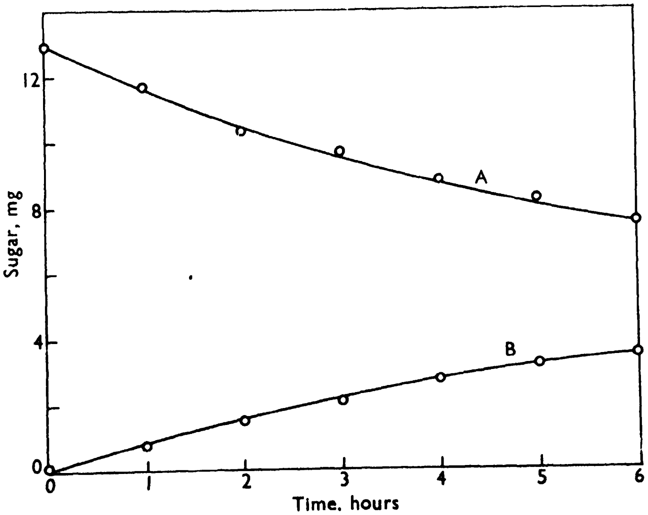

Fig. 2. Progress curves for the hydrolysis of maltose at 37° C: curve A, maltose; curve B, glucose. Reaction mixture comprised 2 ml of a 5 per cent. solution of maltose, 4 ml of buffer solution at pH 5·8 and 2 ml of enzyme solution containing 2 mg of gut tissue enzyme preparation

TABLE II

DETERMINATION OF GLUCOSE AND MALTOSE IN SOLUTIONS AND MIXTURES

The dilution factor for the controls, which were not run through the column, was the same as that for the eluates. The denatured reaction mixture contained buffer salts (pH 5·7) and 0·1 per cent. denatured enzyme preparation

Solutions	Amount of sugar in 4 ml of control, mg	Amount of sugar in 4 ml of eluate, mg
Sugar solutions—		
Glucose	0·430	0·421
Maltose	0·415	0·408
A (1 + 1) mixture—		
Glucose	0·430	0·437
Maltose	0·415	0·405
Denatured enzyme reaction mixtures—		
A (1 + 1) mixture—		
Glucose	0·448	0·456
Maltose	0·420	0·408

For kinetic studies it is essential to remember that appreciable transfer action is possible as the concentration of glucose increases. Generally, it was found that in reaction mixtures containing 12·5 mg per ml of maltose, little transfer activity was measurable below 5 per cent. hydrolysis of the substrate. The change in concentrations of maltose and glucose in such reaction mixtures was readily measured by the technique given.

One of us (D.W.P.) is indebted to the D.S.I.R. for a research scholarship.

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The Detection and Estimation of Aflatoxin in Groundnuts and Groundnut Materials

Part I. Paper-chromatographic Procedure

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A method for the detection and semi-quantitative determination of aflatoxin—a metabolic product of the fungus *Aspergillus flavus* Link—is described.

It is now recognised[1] that the toxicity associated with certain batches of groundnuts is due to the growth on them of a strain or strains of the fungus *Aspergillus flavus* Link ex Fries, which produce, in small amounts, a highly toxic factor to which the name aflatoxin is now being applied.

A provisional chemical test, based on the isolation techniques described by Sargeant, Sheridan, O'Kelly and Carnaghan,[2] has been in use for many months.[3] Subsequent work has, however, shown[4,5] that the toxic factor is often composed of at least two components of different toxicities, and that the paper-chromatographic procedure originally reported[3] does not distinguish between them.

Nesbitt, O'Kelly, Sargeant and Sheridan[6] have recently separated these two components by a counter-current distribution technique and for convenience refer to them as aflatoxin B and aflatoxin G, because of their fluorescent reactions in ultraviolet light when adsorbed on thin layers of aluminium oxide. The same workers[6] report that both components are toxic to day-old ducklings, aflatoxin B exhibiting approximately three times the potency of aflatoxin G.

This paper describes a method for detecting the commoner and more toxic component, aflatoxin B,* together with a procedure permitting the assessment of the likely biological effects of suspect materials.

It must be emphasised that the results and correlations reported in this paper and in Part II of this series are based on the purest sample of aflatoxin B available at the time. It is possible that further work on the isolation of this material may alter somewhat the scale of quantitative values given. It is hoped that these will prove to be at least approximately correct, but, for the present, categories rather than absolute values are preferred for reporting results.

Experimental

During the purification work on aflatoxin it was noticed repeatedly that biological activity of extracts was accompanied by a characteristic blue fluorescence, the presence of which afforded a fairly reliable indication of toxicity on a semi-quantitative basis. This led to the design of the provisional physico-chemical test referred to above.[3]

Chromatographic examinations of material eluted with methanol from the fluorescent spots obtained by this procedure with both alumina (Brockmann activity III) and silica-gel - plaster of Paris (4 + 1) columns have shown[4] that the material in fluorescent spots thus obtained is not homogeneous. Confirmation of this work has been obtained independently by thin-layer chromatography on alumina.[5]

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* Since the work described in this paper and in Part II was carried out, the work of Unilever in Holland (personal communication) has shown that the material designated aflatoxin B can be separated on Kieselgel plates, with a methanol - chloroform system, into two blue fluorescent spots having slightly different R_F values. Of these, apparently, the material isolated from the faster running spot has been shown to be toxic and that from the slower spot to be non-toxic, but potentiating the biological activity of material comprising the faster spot. Hence the tests in these papers describe the detection of a potentiated toxin and it is this material that is described as aflatoxin B. That this is a sufficiently reliable indication of over-all toxicity is, in the authors' opinion, justified, pending further work, since it appears that the proportions of the two components of aflatoxin B in different toxic samples are approximately the same. This is supported by the correlations between total aflatoxin B content determined by these methods and the biological activity exhibited by total extracts of samples examined.

Descending-solvent paper chromatography with the benzene - cyclohexane - methanol - water (5:5:6:4 by volume) system proposed by Rhodes *et al.*[7] on extracts of certain groundnut materials demonstrated the existence of two blue-purple fluorescent components therein. Comparison chromatograms of the purest available material prepared from extracts of toxic groundnut meal and of the purest available material obtained by culture of a strain of *A. flavus* on a synthetic medium (both prepared in this Institute) confirm that the two fluorescent components of the groundnut extracts were aflatoxin B and aflatoxin G.

Unfortunately, the development time for this solvent system is about 6 hours at 20° C, and a more rapid system seemed desirable to reduce the over-all time of analysis. The system benzene - toluene - cyclohexane - ethanol - water (3:3:5:8:5 by volume), to the upper layer of which is added acetic acid so that its concentration is 1 per cent. by volume, has been found efficacious. Descending-solvent development with this system yields a blue-purple fluorescent spot on paper at R_F 0·57 to 0·61, corresponding to aflatoxin B, and from some samples a similar fluorescent spot at R_F 0·28 to 0·31, corresponding to aflatoxin G, is obtained at the same time. The solvent front travels approximately 30 cm at 20° C in 3 hours during development.

The method described below involves extraction of the comminuted groundnut material with methanol, de-fatting the methanol extract with light petroleum (boiling range 40° to 60° C), extraction of the toxin from the de-fatted extract into chloroform and then chromatography of three portions of the purified chloroform extract on paper. Examination of the chromatogram in ultraviolet light (λ 365 mμ) permits the level of toxicity to be classified as very high (>2·0 p.p.m.), high (0·5 to 2·0 p.p.m.) medium (0·1 to 0·5 p.p.m.) or low or negative (<0·1 p.p.m.). These ranges have been established by reference to the chromatographic characteristics and fluorescence phenomena of the purest available sample of aflatoxin B.

During the work described it has been observed repeatedly that the minimum detectable concentration of aflatoxin B on a paper chromatogram is 0·2 μg. A more accurate determination of the toxin content of a sample can be made by the technique of serial dilution until visual extinction of the fluorescent spot occurs. For practical purposes this extinction occurs at the 0·2-μg level, and, from a knowledge of the dilution required to achieve this extinction, the toxin content can be calculated.

METHOD

APPARATUS AND MATERIALS—

The glass apparatus detailed below must be thoroughly cleaned with dichromate mixture and washed free from acid before use. It is convenient to use standard interchangeable-joint glassware when appropriate. "Quickfit" (Quickfit & Quartz Ltd.) apparatus has been found suitable, and the manufacturer's reference numbers are quoted below in brackets.

Soxhlet extractor, 200 ml (EX5/83/200).

Coil condenser, effective length 200 mm (CX3/08).

Boiling flasks, short neck, 250 ml (FR250/3S).

Water bath, 6-hole to accept 250-ml flasks.

Liquid - liquid extractor, downward displacement type, 60 ml (EX10/23).

Disc-baffle distributor, to fit extractor (EX10/20).

Double-surface condenser, effective length 200 mm (CX5/25).

Separating funnels, conical, 500 ml (D83/500).

Chromatographic column, with integral sinter, effective length 200 mm, bore 18 mm (CR32/20).

Calibrated flask, 5 ml.

Graduated micropipette, 100-μl capacity, in divisions of 5 μl.

Graduated micropipette, 25-μl capacity, in divisions of 1·0 μl.

Chromatography tank (22 inches × 12 inches × 7½ inches) equipped with solvent trough and supports (10½ inches).

Chromatography paper, Whatman No. 1, 46 cm × 57 cm.

Extraction thimbles, 41 mm × 123 mm.

Solvents—All solvents specified must be of analytical-reagent grade unless otherwise stated.

Chromatographic alumina—Woelm, neutral.

SAMPLING—

Careful sampling is an essential preliminary to this method, and it is recommended that sampling procedures should follow closely the details laid down by I.U.P.A.C. for oilseeds.[8]

PROCEDURE—

(*a*) *Extraction*—Extract 80 g of finely ground (see Note 1) material with methanol for 6 hours in a 200-ml Soxhlet extractor (syphon rate 4 to 6 cycles per hour). Cool, transfer the methanol extract quantitatively to a 500-ml separating funnel via a small funnel containing a plug of glass-wool, wash the extraction flask with two 10-ml portions of methanol, and add the washings to the contents of the separating funnel. Add 50 ml of light petroleum (boiling range 40° to 60° C) to the contents of the separating funnel, and shake vigorously for 2 minutes. Transfer the lower (methanol) layer to a second 500-ml separating funnel, and repeat the extraction first with a 25-ml portion and then with a 15-ml portion of light petroleum, finally running the methanol layer into a 250-ml boiling flask.

Distil off the methanol at atmospheric pressure, and remove the last traces *in vacuo*. Disperse the fat-free methanol-soluble residue in 60 to 80 ml of water, and extract with chloroform in a downward-displacement-type continuous liquid - liquid extractor for 1½ hours (see Note 2). Reduce the volume of the chloroform extract to about 1 ml by distillation at atmospheric pressure, and place it on a chromatographic column containing neutral alumina (10 g; Brockmann activity I). Elute with 100 ml of chloroform containing 5 per cent. v/v of methanol, concentrate the eluate to small volume, and make up to 5 ml in a calibrated flask with chloroform. Add approximately 0·25 g of anhydrous sodium sulphate to the chloroform solution in the calibrated flask, shake, and allow to settle for 15 minutes.

(*b*) *Chromatography*—Shake the solvent system benzene - toluene - cyclohexane - ethanol - water (3 : 3 : 5 : 8 : 5 by volume) thoroughly in a separating funnel, and set aside overnight in a room at constant temperature (20° C). Separate, and add 1 volume of acetic acid to 100 volumes of upper layer. Use the lower layer in the bottom of the tank, and the acidified upper layer in the trough.

Load 6·25-, 25- and 125-μl portions of the chloroform solution of the toxin prepared and dried as described above on to a baseline 8 cm from the edge of a 23-cm × 50-cm sheet of Whatman No. 1 chromatography paper.

Develop the chromatogram by descending-solvent flow for 3 hours at 20° C, spraying a jet of upper layer solvent just below the origin line on the paper immediately before development, as recommended by Rhodes *et al.* The broad band of saturation produced in the path of development in this manner improves the resolution of the components. At the end of the development period, remove the paper from the tank, mark the position of the solvent front, and dry the paper in air.

(*c*) *Assessment of toxicity levels*—Examine the chromatogram obtained by the above procedure in ultraviolet light (λ 365 mμ), and observe the presence or absence of purple-blue fluorescent spots at R_F 0·57 to 0·61 (aflatoxin B). The toxicity level of the sample under examination may be classified on the basis of the presence or absence of fluorescence at R_F 0·57 to 0·61 after chromatography of the three aliquots referred to above. The classification is shown in Table I.

TABLE I

CLASSIFICATION OF AFLATOXIN B LEVEL

Aliquot from 5 ml, μl	Fluorescence absent, p.p.m. of toxin	Fluorescence observed, p.p.m. of toxin	Aflatoxin B level if fluorescence is observed
6·25	$<$ 2·0	$>$2·0	Very high
25	$<$ 0·5	0·5 to 2·0	High
125	$<$ 0·1*	0·1 to 0·5	Medium

* Samples containing less than 0·1 p.p.m. of toxin are classified as of low or zero aflatoxin B level.

The chromatograms of certain samples may exhibit a second blue-purple fluorescent spot at R_F 0·28 to 0·31, corresponding to aflatoxin G. No sample so far examined has contained this second spot in the absence of aflatoxin B. As aflatoxin G is less toxic than aflatoxin B and is usually present in smaller proportion in naturally toxic groundnuts, the level of toxicity is assessed on the aflatoxin B content.

NOTES—

1. It is recommended that representative samples of decorticated groundnuts be ground before examination in a vegetable slicing and shredding machine until the ground material passes a British Standard 10-mesh sieve.

Representative portions of groundnut meals and cakes should be ground in a laboratory-type hammer mill so that the ground material passes a 10-mesh sieve.

2. The method of extracting the toxin into chloroform described below is suitable when only a few samples have to be analysed.

Place the fat-free aqueous dispersion of the methanol-soluble extract in a 500-ml separating funnel, and saturate with sodium chloride. Extract successively with 100, 50, 25 and 10 ml of chloroform, and combine the chloroform extracts.

RESULTS

The results of the examination of ten typical samples of groundnuts or groundnut products for aflatoxin B by the proposed procedure are shown in Table II, together with the biological test results for extracts of the same samples obtained by Mr. R. B. A. Carnaghan of the Central Veterinary Laboratory, Weybridge.

TABLE II

CORRELATION BETWEEN AFLATOXIN B ASSESSMENT BY THE CHROMATOGRAPHIC PROCEDURE AND BIOLOGICAL ACTIVITY IN THE DUCKLING TEST

			Duckling test	
Sample No.	Product	Aflatoxin B level by chromatographic procedure	Duckling mortality,	Hepatic lesions*
411	Groundnut kernels	Low or zero	0/3	Nil
920	Groundnut kernels	Low or zero	0/3	Nil
571	Groundnut kernels	Low or zero	3/3†	Nil
607	Groundnut kernels	Low or zero	0/3	Nil
498	Groundnut kernels	Medium	0/3	+ to + +
260	Groundnut flour	High	0/3	+ + +
553	Groundnut flour	High	0/3	+ + +
268	Groundnut kernels	Very high	3/3	+ + + +
493	Groundnut kernels	Very high	3/3	+ + + +
552	Groundnut expeller cake	Very high	3/3	+ + + +

* Severity of hepatic lesions is assessed visually on an empirical + to + + + + basis.

† The ducklings in this test were reported to have died as the result of an anaphylactic-type reaction. None of their livers showed lesions characteristic of aflatoxin.

In order to test the efficiency of the method, recovery tests were carried out by adding suitable portions of a methanolic solution of the purest available specimen of aflatoxin B to 20-g portions of an Indian groundnut meal already shown to be toxin-free. Recovery of the toxin by the proposed method gave the results shown in Table III.

TABLE III

RECOVERY OF AFLATOXIN B ADDED TO A NON-TOXIC MEAL

Sample No.	Aflatoxin B level based on toxin added (p.p.m.)		Aflatoxin B level based on toxin recovered
Blank	Zero	(0·0)	Low or zero
1	Low	(0·05)	Low or zero
2	Medium	(0·10)	Medium
3	Medium	(0·50)	Medium
4	High	(1·50)	High
5	Very high	(2·50)	Very high

CONCLUSIONS

At the present time, in the absence of information about the constitution of metabolites produced in groundnuts and their products by the growth on them of strains of *A. flavus*, the method reported provides a semi-quantitative procedure suitable for the detection of toxicity at four levels. The correlation between the analytical results by this method and the biological response in ducklings based on the ten samples tested appears to be satisfactory.

We thank Mr. R. B. A. Carnaghan of the Central Veterinary Laboratory, Weybridge, for the biological test results reported here and Mr. B. J. Francis of this Institute for preparing the extracts for biological testing.

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The Detection and Estimation of Aflatoxin in Groundnuts and Groundnut Materials

Part II. Thin-layer Chromatographic Method

BY J. H. BROADBENT, J. A. CORNELIUS AND G. SHONE

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A semi-quantitative method capable of detecting 0·006 μg of aflatoxin B in groundnuts and groundnut products, especially groundnut meals, by thin-layer chromatography, is described.

THIS paper describes a method more rapid than that proposed by Coomes and Sanders[1] for detecting aflatoxin B in groundnut meals. The de-fatted meal is extracted with methanol,[2] any toxin present in the methanol phase is extracted into chloroform, three aliquots of the chloroform extract are chromatographed on thin layers of aluminium oxide, and the chromatogram is examined in ultraviolet light. The level of aflatoxin B in ground meals can be classified as very high (>2 p.p.m.), high (0·5 to 2·0 p.p.m.), medium (0·1 to 0·5 p.p.m.) and low or zero (<0·1 p.p.m.) on the basis of this procedure. The sizes of the portions required for this classification have been worked out on samples of the purest toxin available.

Aflatoxin G is also separated by the procedure described below and is characterised by a green fluorescence in ultraviolet light; it has a lower R_F value than that of aflatoxin B, which exhibits a purple fluorescence.

The predominant toxic metabolite in naturally infected groundnut materials is aflatoxin B and only in a few extremely highly toxic samples of groundnuts has aflatoxin G been found to be present to any significant extent; hence the method described here deals only with the estimation of aflatoxin B.

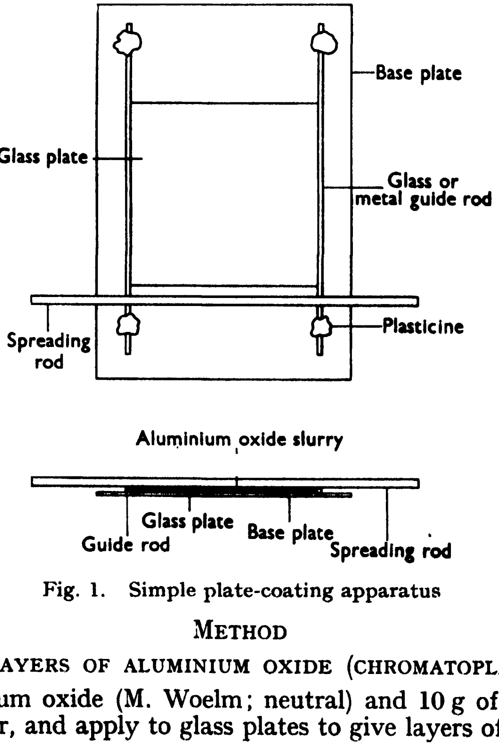

Fig. 1. Simple plate-coating apparatus

METHOD

PREPARATION OF THIN LAYERS OF ALUMINIUM OXIDE (CHROMATOPLATES)—

Mix 90 g of aluminium oxide (M. Woelm; neutral) and 10 g of plaster of Paris into a slurry with distilled water, and apply to glass plates to give layers of thickness 740 ± 10 μ.

Such layers are most conveniently prepared with an apparatus such as the Camag thin-layer chromatography apparatus marketed by Carl Roth of Karlsruhe, Germany (obtainable from T. J. Sas & Son Ltd., London). Alternatively, these layers can be prepared by spreading the slurry with a glass rod on glass plates bounded by glass or metal rods of suitable diameter. The rods may be held on a baseplate with Plasticine (see Fig. 1).

Heat the coated plates in an oven at 100° C for 2 hours, cool, and store in a vacuum desiccator.

Reference compounds should be chromatographed periodically on the prepared chromatoplates to ensure that the activity of the adsorbent is reasonably constant (see Note 1).

EXTRACTION AND CHROMATOGRAPHY OF THE TOXIN—

Extract 20 g (see Note 2) of de-fatted (see Note 3) groundnut meal with analytical-reagent grade methanol for 4 hours (see Note 4) in a 100-ml Soxhlet extractor (syphon rate $\not<$6 litres per hour). Concentrate the extract to 50 ml, and transfer to a separating funnel. Rinse the extraction flask with 25 ml of distilled water, and add the rinsings to the contents of the separating funnel. If an appreciable amount of solid material remains in the flask, add a further 5 ml of water, and transfer to the separating funnel. Wash the flask with 25 ml of chloroform, transfer to the separating funnel, and shake gently; allow to separate, and run off the chloroform layer through a bed of anhydrous sodium sulphate. Repeat the extraction three more times with 25-ml portions of chloroform, and combine the extracts. Concentrate the combined extracts to 35 ml, spot 5- and 20-μl portions (see Note 5) of this concentrate 1·5 cm from one edge of a chromatoplate, and develop with 1·5 per cent. of methanol in chloroform (see Note 6) until a solvent path-length of 10 cm from the base line has been obtained. Examine the resulting chromatogram (see Note 7) in ultraviolet light (λ 365 mμ), and observe the presence or absence of a blue-purple fluorescent spot at R_F 0·5. (The green fluorescence of aflatoxin G occurs at R_F 0·4.) If no fluorescence is visible in this region, concentrate the residual chloroform extract to 5 ml, and chromatograph 15 μl of this concentrate as before. Examine the chromatogram in ultraviolet light (see Note 8).

LEVEL OF AFLATOXIN B—

The aflatoxin B level (see Table I) of the meal being examined may be classified on the basis of the presence or absence of a blue-purple fluorescence at R_F 0·5 after chromatography of the portions referred to above (see Note 9).

It should be noted that a more absolute assessment of aflatoxin B content can be obtained by chromatographing various portions of the chloroform extract and noting the size of the smallest portion giving an observable fluorescence. This portion is then equivalent to about 0·006 μg of aflatoxin B. It will be realised that aflatoxin B levels outside the range given in Table I (0·1 to 2·0 p.p.m.) can be assessed by this technique.

NOTES—

1. It is recommended that the compounds listed below be run periodically with 1·5 per cent. of methanol in chloroform to ensure that the activity of the adsorbent is reasonably constant—

Acridine orange—R_F 0·15.
Phenanthraquinone—R_F 0·90.

Alternatively, a groundnut extract containing aflatoxin B may be used if available (R_F 0·5).

2. Groundnut meals have been found, on occasion, to be non-homogeneous, and care must therefore be taken to ensure representative sampling.

3. Meals that have been produced by expression of the oil from groundnuts will need to be de-fatted by extraction with aromatic-free light petroleum (boiling range 40° to 60° C) or diethyl ether for 2 hours in a 100-ml Soxhlet extractor (syphon rate 10 to 12 changes per hour). If groundnuts are to be tested, they will need to be crushed and de-fatted for a longer period (*e.g.*, 4 hours). Some aflatoxin B (usually less than 1 per cent. of the total amount present) is extracted by light petroleum with the oil. This small amount of aflatoxin B can be recovered if the light petroleum extract is adjusted to about 100 ml and shaken twice with 25 ml of aqueous methanol (25 per cent. v/v). The combined methanol extracts are then added to that obtained from the de-fatted meal.

4. If a rapid indication of whether or not a groundnut meal contains aflatoxin B is required, 20 g of de-fatted meal can be extracted with chloroform for 2 hours,* the extract concentrated to 1 ml, and 20 µl of the concentrate run on a chromatoplate of aluminium oxide. The presence of a blue-purple fluorescent spot at the appropriate position would indicate that the meal contains aflatoxin B at a level greater than 0·1 p.p.m.

5. When portions are being applied to the surface of the chromatoplate the solvent should only be allowed to spread to cover an area defined by 0·5 to 0·7 cm diameter.

6. A chromatographic tank of the smallest convenient size should be used. The sides of the tank should be lined with filter-paper, which should dip into the eluting solvent mixture covering the bottom of the tank (to a depth of 0·5 to 1 cm). Chromatoplates should be developed in the ascending-solvent manner by standing them on the bottom of the tank (resting against one side) with the lower end immersed in the solvent.

7. If more than one spot is to be examined at a time, it is recommended that each spot be viewed individually (beginning with that of least concentration) while the other spots are covered with black paper.

8. Chromatograms should be examined immediately after development, as the fluorescence associated with aflatoxin B fades rapidly.

9. The faintest detectable spot is considered to be a positive in making this assessment.

RECOVERY TESTS

Different amounts of aflatoxin B were added to 20-g samples of a groundnut meal originally shown to be free from toxic material. These artificially fortified samples were de-fatted for 2 hours with aromatic-free light petroleum (boiling rage 40° to 60° C), and the aflatoxin B was recovered by the proposed procedure (see Table II).

TABLE I

AFLATOXIN B LEVEL

Size of portion	Aflatoxin B: No fluorescence observed, p.p.m.	Aflatoxin B: Fluorescence observed, p.p.m.
5 µl from 35 ml	< 2·0	>2·0
20 µl from 35 ml	< 0·5	0·5 to 2·0
15 µl from 5 ml	< 0·1	0·1 to 0·5

TABLE II

RECOVERY OF AFLATOXIN B ADDED TO GROUNDNUT MEAL

Sample No.	Aflatoxin B level based on toxin added (p.p.m.)		Aflatoxin B level based on toxin recovered
Blank	Zero	(0·0)	Low or zero
1	Low	(0·05)	Low or zero
2	Medium	(0·20)	Medium
3	Medium	(0·40)	Medium
4	High	(1·25)	High
5	Very high	(2·50)	Very high

We thank our colleagues for their co-operation, and especially Miss Ann Player and Mr. T. W. Hammonds for their experimental assistance and Messrs. T. J. Coomes and W. S. A. Matthews for use of their aflatoxin B isolate.

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The Extraction of the Lanthanide Elements from Perchloric Acid by Di-(2-ethylhexyl) Hydrogen Phosphate

By T. B. PIERCE AND P. F. PECK

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The elements lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium and yttrium have been extracted from an aqueous perchlorate phase at different acidities into a solution of di-(2-ethylhexyl) hydrogen phosphate in toluene. Separation factors for all the elements of the lanthanide series have been calculated, and the average value for nearest neighbours was found to be 2·40 ± 0·87. The extraction behaviour of yttrium was found to be between those of holmium and erbium.

INVESTIGATIONS into the use of alkyl phosphates as reagents for the solvent extraction of metal ions from aqueous solutions have shown that relatively good separation factors can be obtained for adjacent pairs of lanthanide elements if di-(2-ethylhexyl) hydrogen phosphate (HDEHP) is used as extractant.[1] Since separation factors are not sufficiently large to permit complete isolation of the individual elements with one equilibration, several extractions are necessary, and it has been found that a multistage extraction effect can be achieved simply by retaining the HDEHP on a solid support that has been packed to form a chromatographic column.[2]

Elements of the lanthanide series can be readily extracted from dilute acid solution on to the top of a column of this type, and can then be eluted in the order of increasing atomic number by perchloric acid of gradually increasing strength.

The extraction of the elements lanthanum, promethium, yttrium and thulium by a solution of HDEHP in toluene from a perchlorate medium has already been considered,[3] but elution of the rare earths from HDEHP columns has indicated that the ease of separation of adjacent elements varies appreciably from pair to pair through the series. To assess the value of this difference and the possibility of using the HDEHP - perchloric acid system for rare-earth separation, the extraction of all the lanthanide elements from perchloric acid by a solution of HDEHP in toluene has been investigated. Since considerably different acidities were required to obtain the same distribution ratio for the most widely different members of the series, lanthanum and lutetium, no attempt was made to maintain the perchlorate concentration of the aqueous phase constant, and perchlorate solutions were made up by diluting concentrated perchloric acid to the appropriate value.

EXPERIMENTAL

The method used to purify the HDEHP was based on that described elsewhere.[1] After this purification, samples of HDEHP from two different manufacturers were found to give the same distribution ratio under similar conditions for extraction of the rare earths. Before use, 2 ml of the acid were diluted to 10 ml with redistilled toluene. Each of the two phases brought into contact during the extraction process was always pre-equilibrated with the other before use, at the temperature at which equilibrations were carried out, *i.e.*, 25° ± 0·1° C. Ten millilitres of both solutions required for the equilibration were transferred by pipette to a centrifuge tube, the temperature of which was thermostatically controlled, and active lanthanide tracer was added in not more than 0·01 ml of solution. The phases were then mixed by stirring briskly for 20 minutes by means of a glass stirrer connected to an electric motor, separated by centrifugation, and 2 ml of each solution were carefully removed by pipette to permit the distribution of the radioactivity to be assayed. The concentration of acid in the aqueous phase was determined by titration against standard alkali.

To confirm that equilibrium had been attained, organic phases containing extracted tracer were re-equilibrated with fresh perchloric acid to allow equilibrium to be reached from conditions of metal-deficient, as well as metal-rich, aqueous phase.

Rare-earth tracers were obtained by irradiating Specpure chemicals in a neutron flux of 10^{12} neutrons per sq. cm per second, and, after irradiation, the γ-ray spectra of these tracers were examined for interfering radiations. Interference from impurities or daughter products was avoided by careful choice of irradiation and decay times, by the use of γ-ray spectrometry or by chemical separation of the required lanthanides. When chemical separations were necessary, these were carried out by elution of the rare earths with perchloric acid at 60° C from columns of HDEHP retained on a poly(vinyl chloride - vinyl acetate) copolymer.[2]

A solution of promethium in perchloric acid was prepared by adsorbing several milligrams of irradiated neodymium, together with daughter promethium, on to an HDEHP column, and then eluting with 0·40 M perchloric acid, when first the neodymium and then the promethium appeared in the effluent. γ-Ray spectrometry showed that there was no detectable neodymium activity in the solution of promethium.

Experimental results were plotted on a graph of the logarithm of the distribution ratio (logD) against logarithm of the hydrogen ion concentration of the aqueous phase ($\log[H^+]$), and the best straight lines through these points were found by the method of least squares. The slope of this line, and the intercept at $D = 1$, were also calculated.

RESULTS AND DISCUSSION

The distribution of certain lanthanide elements between perchloric acid and a solution of HDEHP in toluene has been shown to be dependent on the third power of both the hydrogen ion and free reagent concentration, reaction being considered to occur between the tervalent metal ion and an HDEHP dimer,[3] thus—

$$M^{3+} + 3\,(\mathrm{HDEHP})_2 \rightleftharpoons M\{\mathrm{H(DEHP)_2}\}_3 + 3\mathrm{H}^+$$

The third power dependency of the distribution ratio on reagent concentration was confirmed by extraction of europium from a 0·47 M solution of perchloric acid in experiments not reported here. Since the ionic strength of the aqueous phase was not maintained constant during the extraction of each element, some deviation of the slope of the plot of logD against

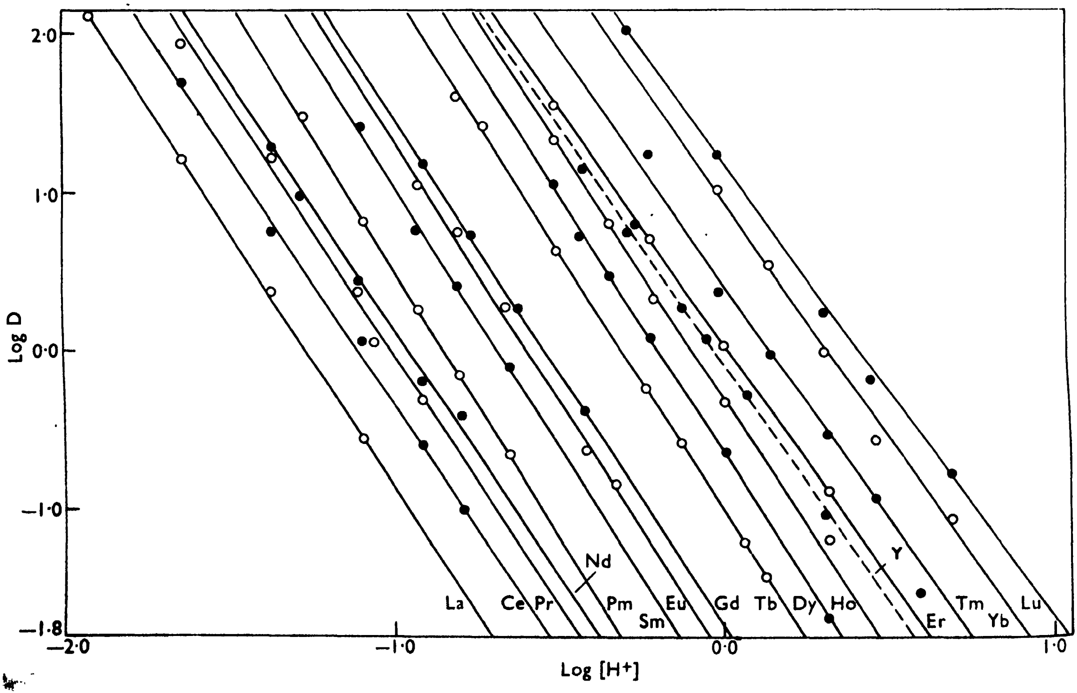

Fig. 1. Extraction of the lanthanides and yttrium from an aqueous perchloric acid phase by a solution of HDEHP in toluene

$\log[H^+]$ from the ideal value of 3 could be anticipated, and calculation showed that the mean slope of the lines for all the lanthanides and yttrium, obtained by the method of least squares, was 3·16 ± 0·15. The experimental results, from which this value is derived, are shown in Fig. 1, in which lines are drawn to fit the slope and intercept calculated for each element.

To assess the ease with which any pair of rare earths can be separated from each other, separation factors were calculated from intercepts of the logD against $\log[H^+]$ plots for all the elements with the axis logD = 0, that is to say at the point where 50 per cent. of the metal is extracted. These results are shown in Table I, the separation factor $S_{1,2}$ for metals 1 and 2 being given by $S_{1,2} = [H]_1^3/[H]_2^3$, the acidities referring to the hydrogen ion concentration at the point D = 1. When trying to obtain complete isolation of one rare earth from all others by extraction with HDEHP, the most troublesome decontamination of any element is likely to be from its nearest neighbours. Thus the separation factor for adjacent pairs of rare earths provides a means for assessing the ease of carrying out the most difficult decontamination step, and Peppard *et al.* have shown with a number of lanthanides that, in the 0·75 M HDEHP - 0·5 M hydrochloric acid system, an approximately linear relationship exists between the logarithm of the distribution ratio and the atomic number (*Z*) of an element, the average separation factor for adjacent lanthanides being about 2·5.[1] A graph of the function $3 \log[H^+]$ at logD = 0, against atomic number *Z*, is shown in Fig. 2 for the HDEHP - perchloric acid system for all the elements from *Z* = 57 to *Z* = 71, from which it is seen that a straight line plot is once more obtained, the average separation factor for nearest neighbours being 2·40 ± 0·87, which agrees well with the value obtained when extractions were carried out from a hydrochloric acid phase.[1] The "apparent" atomic number of yttrium calculated on the assumption that the $3 \log[H^+]$ value for yttrium at logD = 0 falls on the line shown in Fig. 2 was found to be 67·5, this being similar to the value of 67·6 previously found for the HDEHP - hydrochloric acid system.[1]

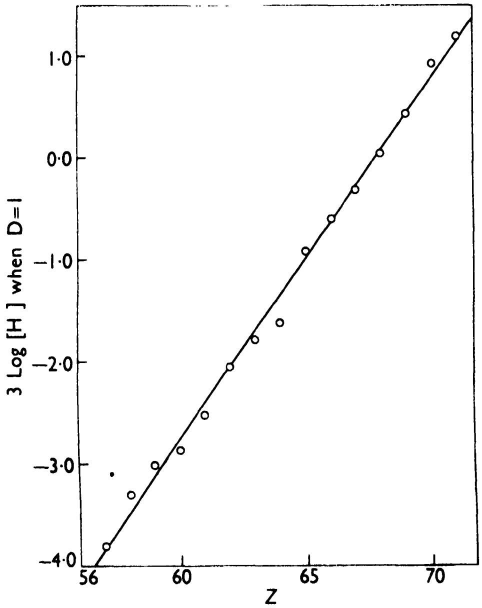

Fig. 2. Dependence on atomic number (*Z*) of acidity for 50 per cent. extraction of the lanthanides

It has already been pointed out that the average separation factor calculated for adjacent rare earths in the HDEHP system compares favourably with that found when tributyl phosphate is used as organic reagent,[1] but it can be seen from Table I that considerable deviation from the average separation factor of 2·4 occurs throughout the series, the values lying between $S_{Tb - Gd} = 4·93$ and $S_{Nd - Pr} = 1·38$. Further, if an over-all separation factor

Cerium	Praseodymium	Neodymium	Promethium	Samarium	Europium	Gadolinium	Terbium	Dysprosium	Holmium	Yttrium	Erbium	Thulium	Ytterbium	Lutetium	
2·98	6·09	8·42	1·82 $\times 10^{1}$	5·54 $\times 10^{1}$	1·05 $\times 10^{2}$	1·51 $\times 10^{2}$	7·43 $\times 10^{2}$	1·56 $\times 10^{3}$	3·03 $\times 10^{3}$	5·00 $\times 10^{3}$	6·84 $\times 10^{3}$	1·70 $\times 10^{4}$	5·25 $\times 10^{4}$	9·78 $\times 10^{4}$	Lanthanum
	2·05	2·93	6·11	1·86 $\times 10^{1}$	3·54 $\times 10^{1}$	5·07 $\times 10^{1}$	2·50 $\times 10^{2}$	5·26 $\times 10^{2}$	1·02 $\times 10^{3}$	1·68 $\times 10^{3}$	2·30 $\times 10^{3}$	5·71 $\times 10^{3}$	1·76 $\times 10^{4}$	3·29 $\times 10^{4}$	Cerium
		1·38	2·98	9·11	1·73 $\times 10^{1}$	2·48 $\times 10^{1}$	1·22 $\times 10^{2}$	2·57 $\times 10^{2}$	4·98 $\times 10^{2}$	8·22 $\times 10^{2}$	1·12 $\times 10^{3}$	2·79 $\times 10^{3}$	8·62 $\times 10^{3}$	1·61 $\times 10^{4}$	Praseodymium
			2·16	6·58	1·25 $\times 10^{1}$	1·79 $\times 10^{1}$	8·82 $\times 10^{1}$	1·86 $\times 10^{2}$	3·60 $\times 10^{2}$	5·49 $\times 10^{2}$	8·12 $\times 10^{2}$	2·02 $\times 10^{3}$	6·23 $\times 10^{3}$	1·16 $\times 10^{4}$	Neodymium
				3·05	5·80	8·30	4·09 $\times 10^{1}$	8·61 $\times 10^{1}$	1·67 $\times 10^{2}$	2·75 $\times 10^{2}$	3·76 $\times 10^{2}$	9·36 $\times 10^{2}$	2·89 $\times 10^{3}$	5·38 $\times 10^{3}$	Promethium
					1·90	2·72	1·34 $\times 10^{1}$	2·82 $\times 10^{1}$	5·47 $\times 10^{1}$	9·02 $\times 10^{1}$	1·23 $\times 10^{2}$	3·07 $\times 10^{2}$	9·46 $\times 10^{2}$	1·76 $\times 10^{3}$	Samarium
						1·43	7·04	1·48 $\times 10^{1}$	2·88 $\times 10^{1}$	4·74 $\times 10^{1}$	6·48 $\times 10^{1}$	1·61 $\times 10^{2}$	4·98 $\times 10^{2}$	9·28 $\times 10^{2}$	Europium
							4·93	1·04 $\times 10^{1}$	2·01 $\times 10^{1}$	3·32 $\times 10^{1}$	4·53 $\times 10^{1}$	1·13 $\times 10^{2}$	3·48 $\times 10^{2}$	6·49 $\times 10^{2}$	Gadolinium
								2·11	4·08	6·73	9·21	2·29 $\times 10^{1}$	7·06 $\times 10^{1}$	1·32 $\times 10^{2}$	Terbium
									1·94	3·20	4·37	1·09 $\times 10^{1}$	3·35 $\times 10^{1}$	6·25 $\times 10^{1}$	Dysprosiur
										1·65	2·25	5·61	1·73 $\times 10^{1}$	3·22 $\times 10^{1}$	Holmium
											1·37	3·40	1·05 $\times 10^{1}$	1·96 $\times 10^{1}$	Yttrium
												2·49	7·67	1·43 $\times 10^{1}$	Erbium
													3·09	5·75	Thulium
														1·86	Ytterbium

TABLE I

SEPARATION FACTORS FOR THE RARE EARTHS CALCULATED FOR THE PERCHLORIC ACID - HDEHP SYSTEM of 10^4 is required to obtain virtually complete separation of two elements by a single liquid - liquid extraction, it is clear from Table I that rare earths whose atomic numbers differ by less than about 12 units cannot be separated by a single extraction with HDEHP; a multistage-extraction procedure is therefore required.

Wide use has been made of ion-exchange resins for rare-earth separations, the elements being eluted chromatographically from a column of cation-exchange resin by a solution of complexing agent chosen to increase separation. The average separation factors for elution of the lanthanides from Dowex 50 by glycolate at 87° C, by α-hydroxyisobutyrate at 87° C and by lactate at 20° C are 1·43, 1·62 and 1·51, respectively,[4] which are much smaller than the value of 2·4 obtained for the HDEHP system, but useful separations are achieved as chromatographic elution provides a means of obtaining a multistage extraction effect. If it is inconvenient to utilise conventional equipment to provide the large number of liquid - liquid extraction stages necessary to exploit the high separation factors for the rare earths obtained by extraction with HDEHP, it can therefore be seen that an alternative method would be to incorporate the HDEHP in a partition-chromatographic system. By this type of technique HDEHP has been employed for the separation of the lanthanides on the laboratory scale without the use of large pieces of solvent-extraction equipment.[5]

We thank Mr. R. Hallett for providing a computer programme for the method of least squares.

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The Paper Chromatography of Some Substituted Cinnolines with Aqueous Solvents

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The chromatography of cinnolines in six solvents is described. The 4-methylcinnolines migrated satisfactorily in all solvents. None of the solvents was suitable for the chromatography of the 4-methylcinnoline quaternary compounds. Satisfactory separation of the 4-hydroxycinnolines is possible only in those solvents in which tautomerism is prevented. The role of intramolecular and intermolecular hydrogen bonding in the chromatography of the 4-hydroxycinnolines has been discussed.

AQUEOUS solvents have been used by Albert and co-workers[1,2,3,4,5] for separating pteridines, purines and pyrazines on paper, and by Nicolaus[6] for thin-layer chromatography of pteridines. In this paper the separation of cinnolines on paper with aqueous solvents is described for the first time.

EXPERIMENTAL

The compounds were chromatographed on Whatman No. 1 papers (19·5 cm × 19·5 cm) by an ascending-solvent technique, in the machine direction of the paper fibres, with six solvents.

(1) Ammonium chloride solution, 5 per cent. w/v.
(2) A (1 + 1) mixture of 5 per cent. w/v ammonium chloride solution and 5 per cent. v/v ammonium hydroxide.
(3) Ammonium hydroxide, 1 per cent. v/v.
(4) Ammonium hydroxide, 5 per cent. v/v.
(5) Ammonium hydroxide, 10 per cent. v/v.
(6) Ammonium hydroxide, 25 per cent. v/v.

Portions (0·0025 ml) of 1 per cent. solutions of the compounds were applied at 2-cm intervals, 6 cm from the base of the filter-paper. The dispersing solvents were removed by a current of warm air.

The prepared papers were equilibrated with solvent vapour for 1 hour, after which they were eluted for 1 hour at a constant temperature of 25° ± 0·5° C; the solvent front rose by 18 cm.

The dried papers were examined under an ultraviolet lamp. The compounds appeared as dark or coloured spots on a blue fluorescent background.

RESULTS

The R_F values, the mean of 5 tests, reproducible to $\pm 0{\cdot}02$ R_F unit, are shown in Table I.

DISCUSSION

4-METHYLCINNOLINES—

These gave discrete measurable spots in all solvents. Their R_F value increased with increased ammonium hydroxide concentration of the solvent, reaching a maximum value in solvent No. 5; the 7-carboxylic acid ethyl ester alone shows an increased R_F value in solvent No. 6.

The differences in R_F values for the isomeric 6-chloro- and 7-chloro- compounds are too small for them to be separated by any of the solvents. The 7-carboxylic acid and its ethyl ester are separable in all solvents.

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TABLE I

R_F VALUES FOR SUBSTITUTED CINNOLINES

Compound	R_F value in Solvent No.— 1	2	3	4	5	6	Fluorescence
4-*Methylcinnolines*—							
6-Chloro-	0·45	0·47	0·53	0·54	0·57	0·57	Dark
7-Chloro-	0·48	0·52	0·54	0·55	0·59	0·59	Dark
7-Carboxylic acid..	0·58	0·68	0·77	0·86	0·87	0·87	Dark
7-Carboxylic acid ethyl ester ..	0·47	0·49	0·55	0·57	0·61	0·66	Dark
Quaternised 4-*methylcinnolines*—							
1-Ethiodide-	0·00	C	C	C	C	C	Dark
6-Chloro-1-ethiodide-	0·00	C	C	C	C	C	Dark
7-Chloro-1-methiodide-	0·00	C	C	C	C	C	Dark
7-Chloro-1-ethiodide-	0·00	C	C	C	C	C	Dark
7-Carboxylic acid-1-ethiodide- ..	0·00	C	C	C	C	C	Dark
4-*Hydroxycinnolines*—							
7-Chloro-	C	C	C	C	0·66	0·71	Dark
6,7-Dichloro-	C	C	C	C	0·33	0·52	Dark
6-Chloro-7-methyl-	C	C	C	0·54	0·66	0·73	Dark
6-Bromo-7-methyl-	C	C	C	0·41	0·53	0·60	Dark
8-Methyl-6-nitro-	C	C	C	0·61	0·72	0·77	Dark
7-Methyl-6-nitro-	C	C	C	0·42	0·55	0·60	Dark
3-Ethyl-	C	C	C	0·52	0·75	0·81	Yellow
7-Chloro-8-nitro-	C	C	C	0·42	0·54	0·62	Dark
3-Methyl-8-nitro-	C	C	C	C	0·42	0·52	Yellow
7-Methyl-8-nitro-	C	C	C	C	0·72	0·76	Dark

C = Comet-shaped spots unsuitable for measurements.

4-Methylcinnoline quaternary compounds—

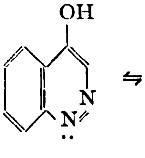
These compounds, quaternised on the basic-1-nitrogen atom,[7] gave comet-shaped spots in solvents Nos. 2 to 6; the tail followed the direction of solvent flow, the head remaining on the point of application. Since these compounds are decomposed, reforming the base, in alkaline solutions,[8] tailing may be caused by such decomposition and the resulting separation of the decomposition products. The absence of tailing in solvent No. 1 and the increased tail length with increased alkalinity of the solvent supports this view.

Solvent No. 1 is suitable for the separation of the 4-methylcinnolines from their corresponding quaternary compounds.

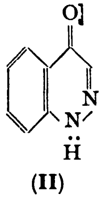
4-Hydroxycinnolines—

An increase in the alkalinity of the solvent resulted in higher R_F values for these compounds. This is to be expected because of their acidic properties.[9,10]

All gave comet-shaped spots in one or more of the solvents, the tail being against the direction of solvent flow. Ultraviolet absorption spectroscopy[11] has shown the existence of two tautomers, the phenolic form (I) and the cinnolone form (II). The tailing of the spot may be attributed to the partial separation of the tautomers.

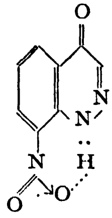

(I)

⇋


(II)

Franc[12] has related the energy content of the intermolecular hydrogen bond formed between a compound and the stationary phase to the R_F value. Bonds of type (*a*) O . . .HO have a higher energy content than bonds of type (*b*) N . . . HO, and compounds forming bonds of type (*a*) have lower R_F values than those forming bonds of type (*b*). Thus the comet head is probably the cinnolone tautomer, since this would form type (*a*) bond with the cellulose surface. The tail preceding the spot consists of the phenolic tautomer that forms the weaker type (*b*) bond. The reduction in the size of the comet head, and the reduction and final elimination of the tail, with increased alkalinity of the solvent is a consequence of the stabilisation of the compound in the phenolic form as a result of salt formation.

In the 4-hydroxy-8-nitro-compounds, the cinnolone form is stabilised by intramolecular hydrogen bonding (III).[13] This results in these compounds giving discrete spots in solvents Nos. 5 and 6 only. That the 7-chloro-4-hydroxy-8-nitrocinnoline gave a discrete spot in solvent No. 4 as well is probably caused by the electron withdrawing the chlorine atom adjacent to the nitro-group reducing the electron density on the chelating oxygen atom and thus weakening the intramolecular hydrogen bond.



(III)

The separation of isomers is possible in those solvents in which discrete spots are obtained. Thus the 4-hydroxyl-8-methyl-6-nitrocinnoline and the corresponding 7-methyl compounds are separable, as are the 4-hydroxy-3-methyl-8-nitrocinnoline and the corresponding 7-methyl compound. The 6-halogeno-4-hydroxy-7-methyl compounds are also separable.

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Qualitative Examination of Detergents by Paper Chromatography

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Paper chromatography can be used for separating and identifying the main organic constituents of commercial detergents. These are anionic, cationic and non-ionic surface-active agents, soaps, toluene- and xylene-sulphonates, alkanolamines and urea. By a method of consecutive spraying, all these substances can be detected on a single chromatogram. The presence of metallic cations can also be shown on this chromatogram, but, to differentiate between the metals, a second chromatogram must be developed with a different solvent. This solvent is also useful for separating amines from amide hydrolysate.

THE work so far reported on paper chromatography of detergents deals mainly with the separation of members of homologous series. Holness and Stone[1,2] separated the homologues of alkyl sulphates, alkylpyridinium sulphates, alkyltrimethylammonium halides and alkylbenzyldimethylammonium halides. Franks[3,4] reported similar separations obtained by reversed-phase chromatography on paper treated with cetyl alcohol; he used aqueous ethanol as the mobile phase. He described an elaborate method for continuous variation of the mobile phase during the development of the chromatogram. Borecký[5] claimed to have improved the methods of the above-mentioned authors by using paper treated with lauryl alcohol and developing with mixtures of methanol, ammonia and formic acid. Longman and Hilton[6] described the use of paper impregnated with ammonium acetate for separating mixtures of homologous fatty acid alkanolamides.

Gallo[7] investigated the effect of various solvents on the rate of migration of non-ionic detergents on paper, and Ginn, Church and Harris[8] used a paper-chromatographic method for detecting and determining polyglycol in non-ionic detergents.

EXPLORATORY WORK

COLOUR REAGENTS—

Our experiments on paper chromatography began several years ago; bromocresol green was used in Heinerth and Pollerberg's[9] method for detecting and identifying alkanolamines in commercial liquid detergents and shampoos. We used Whatman No. 541 filter-paper and developed for 4 hours with the recommended solvent—isopropanol - water - ammonia (100 + 18 + 2). Many samples gave blue spots near the origin, and these spots were subsequently shown to be due to alkali metals. Further experiments showed that polyethoxylates and urea could also be revealed on these same chromatograms by spraying first with cobalt thiocyanate solution and then with *p*-dimethylaminobenzaldehyde.[10]

The use of cobalt thiocyanate for detecting polyethoxylates in solution was described by van der Hoeve[11,12] and other workers.[13,14] The test proved to be much more sensitive on paper than in a tube, especially for substances containing short ethylene oxide chains and for sulphated ethoxylates. The Dragendorff reagent, as used by Gallo[7] and Ginn, Church and Harris,[8] was tried, but the cobalt thiocyanate was better for sulphated ethoxylates. Both sprays also give colours with cationic detergents. Recently, we found that the iodine treatment suggested by Barrett[15] would detect polyethoxylates and cationic surface-active agents. The method is useful in that it leaves the paper unchanged after the iodine disappears.

Spraying with pinacryptol yellow, as used by Holness and Stone[1] and Borecký,[5,16] or with rhodamine B, as suggested by Longman and Hilton,[6] showed that both reagents gave colours in ultraviolet light with anionic, cationic and non-ionic detergents. The clearest colours were given by a mixture of the dyestuffs, but unfortunately this mixture gave with soaps orange colours similar to those given with anionic detergents. Differentiation between these types of substance and clarity of the colours could both be obtained by using first pinacryptol yellow and then rhodamine B.

SOLVENTS—

When Heinerth and Pollerberg's solvent was used, most surface-active agents did not separate, but travelled with the solvent front, thus limiting the usefulness of the colour reagents. Many other solvents were tested in an attempt to obtain separation, and, up to the present, the best results have been given by a mixture of t-butanol, water and ammonia (100 + 6 + 6). With this solvent a single chromatogram on Whatman No. 1 filter-paper achieves not only a separation of ionic from non-ionic detergents, but also a better separation of alkanolamines than that obtained by Heinerth and Pollerberg, and a separation of toluene sulphonates from xylene sulphonates. The only slight disadvantage is the long development period of 16 to 17 hours. Garcia and Couerbe's[17] solvent—benzene - pyridine - acetic acid - water (80 + 80 + 2 + 8)—and various modifications of it gave partial success by separating non-ionic (R_F 0·95) from ionic detergents (R_F about 0·6), but both types tended to leave trails. All other substances stayed at the origin.

To differentiate between metals we used Martin's[18] solvent—methanol - ammonia (90 + 10)—which also was useful for separating alkanolamines and taurines obtained by hydrolysis of amides.

TECHNIQUE—

All work was done by ascending-solvent chromatography, because of the simplicity of the method. Washing of the paper was essential for separating metals and gave cleaner results in the general examination of detergents, although it was not absolutely necessary for this.

Spots of sample solutions were applied to the paper by means of a loop of Nichrome wire, which was cleaned by heating to redness after each application. A micropipette could be used, but it could not be cleaned so easily as the wire. To identify components we used marker solutions containing known substances, and avoided relying on R_F values, which were liable to variations depending on, among other things, the water content of the paper. Partial drying of the paper by controlled heating in an oven before developing the chromatogram was important for good separation of the non-ionic from the ionic detergents. Omission of heating caused all the surface-active agents to travel with the solvent front, whereas complete removal of moisture made all substances, except the non-ionic detergents, stay at the origin. The degree of heating required in any particular circumstances must be determined by experiment.

TABLE I

DETERGENT COMPONENTS STUDIED ON CHROMATOGRAMS

Substance	Formula*	Usual values of—	
		x	*n*
Anionic surface-active agents—			
Alkylarylsulphonates	$C_xH_{2x+1}.C_6H_4.SO_3M$	12	—
Alkyl sulphates	$C_xH_{2x+1}.O.SO_3M$	12 to 18	—
Sulphated alcohol ethoxylates	$C_xH_{2x+1}.[OC_2H_4]_n.O.SO_3M$	12 to 18	1 to 4
Sulphated alkylphenol ethoxylates	$C_xH_{2x+1}.C_6H_4.[OC_2H_4]_n.O.SO_3M$	8 to 9	1 to 4
Taurides	$C_xH_{2x+1}.CO.N(CH_3 \text{ or } H).C_2H_4.SO_3M$	11 to 17	—
Sarcosinates	$C_xH_{2x+1}.CO.N(CH_3).CH_2.COOM$	11 to 17	—
Sulphosuccinates	$C_xH_{2x+1}.O.CO.CH_2.CH(SO_3M).COO.C_xH_{2x+1}$	8	—
Non-ionic compounds—			
Monoethanolamides	$C_xH_{2x+1}.CO.NH.C_2H_4OH$	11	—
Diethanolamides	$C_xH_{2x+1}.CO.N(C_2H_4OH)_2$	11	—
Ethoxylated monoethanolamides	$C_xH_{2x+1}.CO.NH.C_2H_4.[OC_2H_4]_n.OH$	11	2 to 10
Ethoxylated alcohols	$C_xH_{2x+1}.[OC_2H_4]_n.OH$	12 to 18	8 to 12
Ethoxylated alkylphenols	$C_xH_{2x+1}.C_6H_4.[OC_2H_4)_n.OH$	8 to 9	8 to 12
Other compounds—			
Quaternaries	$C_xH_{2x+1}.N(CH_3)_3.X$	12 to 18	—
Amine oxides	$C_xH_{2x+1}.N(CH_3)_2.O$	12 to 18	—
Toluene sulphonates	$CH_3.C_6H_4.SO_3M$	—	—
Xylene sulphonates	$(CH_3)_2.C_6H_3.SO_3M$	—	—

* M = sodium, potassium, ammonium or alkanolamine.
X = halide or sulphate.

The technique of consecutive spraying, although not so good as that in which a separate paper is used for each spray, gave the required information without necessitating the development of many chromatograms.

The detergent components studied are listed in Table I.

METHOD

Three procedures are described. The first is the general procedure for examining detergents, the second is for separating metals and the third for identifying amines produced by hydrolysis of fatty acid alkanolamides and taurides.

GENERAL REAGENTS—

Hydrochloric acid, concentrated.

Ethanol—Industrial methylated spirit may be used.

Iodine crystals.

DEVELOPING SOLVENTS—

Butanol solvent (for the main analysis)—

t-Butanol	100 ml
Ammonia solution, sp.gr. 0·88 ..	6 ml
Water	6 ml.

Methanol solvent (for separating metals and amines)—

Methanol	90 ml
Ammonia solution, sp.gr. 0·88 ..	10 ml.

MARKER SOLUTIONS—

Marker A—Dissolve 0·25 g of mono-, di-and triethanolamine and 1 g of urea in a mixture of 50 ml of water and 50 ml of ethanol.

Marker B—Dissolve 0·25 g of sodium toluene sulphonate in a mixture of 50 ml of water and 50 ml of ethanol.

Marker C—Dissolve 0·25 g of sodium chloride, 0·25 g of potassium chloride and 0·25 g of magnesium chloride in 50 ml of water. Add 50 ml of ethanol, and mix.

SPRAY SOLUTIONS—

Pinacryptol yellow—Dissolve 0·05 g of pinacryptol yellow (obtainable from G. T. Gurr Ltd., 136–138, New Kings Road, London, S.W.6) in 100 ml of ethanol. Filter before use.

Rhodamine B—Dissolve 0·01 g of rhodamine B in 100 ml of ethanol. Filter before use.

Bromocresol green—Dissolve 0·04 g of bromocresol green in 100 ml of ethanol. Add dilute ammonia solution dropwise until the yellow colour is just changing to green.

Cobalt thiocyanate—Prepare a stock solution by dissolving 1·4 g of cobalt nitrate crystals and 87 g of ammonium thiocyanate in water, and dilute to 500 ml. Prepare the spray solution by mixing 50 ml of stock solution with 50 ml of ethanol.

p-*Dimethylaminobenzaldehyde*—Dissolve 1 g of *p*-dimethylaminobenzaldehyde in 50 ml of N hydrochloric acid, and then add 50 ml of ethanol.

All of the spray solutions described above may be kept for several weeks.

Sprays for amines—Prepare each just before use.

(*a*) Mix 20 ml of M sodium carbonate and 2 ml of acetaldehyde.

(*b*) Dissolve 0·2 g of sodium nitroprusside in 20 ml of water.

SPECIAL APPARATUS—

Whatman No. 1 *paper for chromatography.*

Two battery jars approximately 15 cm in diameter and 30 cm high with close-fitting lids. One is used for developing the chromatograms. A few crystals of iodine are placed in a small beaker in the bottom of the second (see p. 228).

A 5-cm length of platinum or Nichrome wire with one end formed into a 3-mm diameter loop and the other end sealed in a short length of glass tubing. The plane of the loop must be at right angles to the rest of the wire. A loop of this size transfers 5 to 10 μl of solution to the paper.

Chromatographic sprays.

Ultraviolet lamp.

PROCEDURE FOR GENERAL EXAMINATION OF DETERGENTS—

Development of Chromatogram

Wash a sheet of Whatman No. 1 paper (30 cm × 28 cm) in a large flat dish with approximately 0·2 N hydrochloric acid, then four times with distilled water and finally with 50 per cent. ethanol. The addition of ethanol to the final wash makes the paper easier to handle without tearing and expedites drying. Hang the paper up to dry at room temperature. Several sheets may be washed together, but they should be hung up singly to dry.

Dilute 1 ml of liquid detergent sample to about 20 ml with 50 per cent. ethanol before application to the paper. If inorganic matter separates, it can be ignored. When the sample is a solid, dissolve about 0·2 g in 20 ml of 50 per cent. ethanol. The degree of dilution desirable depends on the concentration of the sample. A high concentration of surface-active matter in a spot may cause distortion of the solvent front with consequent uneven development of the chromatogram, but, if a sample is diluted too much, minor components may not be detected. When space is available, use a sample at several dilutions.

Draw a line in pencil 3 cm from one long side of the paper, and apply spots of marker solutions A and B and of the diluted sample solutions to this line at 2·5-cm intervals. Label each spot in pencil. Allow the spots to dry at room temperature, and then heat the paper in an oven at 80° C for 90 seconds to remove some of the moisture from the paper.

Put about 100 ml of the t-butanol solvent in one of the battery jars. The solvent may be used for about six runs before it needs to be changed. Form the paper into a cylinder, fasten it at the top with a paper clip, and place it in the jar. Cover the jar, and leave it overnight at 15° to 20° C until the solvent front on the paper has risen about 20 cm above the base line. This takes 16 to 18 hours. If the temperature falls below 15° C, the solvent becomes so viscous that it does not rise far on the paper. Dry the paper in the oven at 80° C.

Spraying

Pinacryptol yellow—Spray the paper evenly with the pinacryptol yellow solution, allow to dry at room temperature, and examine in ultraviolet light. Draw pencil lines around all visible spots, and record their colours.

Heat the paper for 2 to 3 minutes in an oven at 80° C, and examine again in ultraviolet light. Observe any changes in colour of the spots.

Rhodamine B—Spray the paper with rhodamine B solution, and again examine in ultraviolet light both before and after heating.

Iodine—Stand the paper for about one minute in the battery jar containing iodine crystals. Examine the paper for brown spots, and observe whether or not these spots fade in a few minutes. In ultraviolet light the spots are prominent as dark green areas.

Bromocresol green—Spray with bromocresol green solution, and dry at 80° C. Mark all blue spots that appear, because these will be lost in a subsequent spraying with an acid solution. The bromocresol green solution itself quenches all fluorescence in ultraviolet light.

Cobalt thiocyanate—Spray with cobalt thiocyanate solution. Do not heat the paper after this spray has been applied, but allow it to dry at room temperature. Look for bluish green spots. Sometimes faint spots show up better on the back of the paper. The colours due to this spray are often clearer after the *p*-dimethylaminobenzaldehyde spray has been applied because, being acid, it removes all blueness due to bromocresol green.

p-*Dimethylaminobenzaldehyde*—Spray with *p*-dimethylaminobenzaldehyde solution. Allow the paper to dry at room temperature, and look for bright yellow spots.

Interpretation

Pinacryptol yellow—Non-ionic detergents (R_F 0·9 to 0·95) appear as pale blue spots just below the solvent front. Monoethanolamides are often hard to detect. Amine oxides (R_F 0·85), which are rarely met, also show as pale blue spots, but a little lower on the paper.

Most anionic detergents (R_F 0·75) show up as brilliant orange spots that are usually elongated. Some of the colours fade to yellow or blue on prolonged irradiation. Sulphated alkylphenol ethoxylates give violet spots. Soaps (R_F 0·75) form pale yellow spots that fade rapidly.

Cationic detergents (R_F 0·7) show up as pale blue spots that again are usually elongated. Toluene sulphonates (R_F 0·25) form reddish orange spots and xylene sulphonates (R_F 0·32) yellow ones. Marker solution B will show the position of the toluene sulphonates.

Ethanolamines and metallic cations usually appear as faint grey-violet spots, but it is not necessary to look for them at this stage because they are made much more definite with subsequent sprays.

Heating the paper usually changes the colours formed by anionic detergents. Prolonged irradiation with ultraviolet light has a similar effect, probably due to heating by the lamp. Alkylarylsulphonates stay orange and sulphated alkylphenol ethoxylates violet. Spots due to alkyl sulphates change from orange to yellow, often with a blue centre, whereas those due to sulphated alcohol ethoxylates become blue all over. Less common substances, such as taurides, sarcosinates and sulphosuccinates, also change to yellow or blue. Soaps usually remain invisible after the initial fading, but they too may give blue spots. The colours and changes depend to some extent on the concentration of the substances in the spots, and so are only an approximate guide to identification (see Table II).

TABLE II

COLOUR REACTIONS IN ULTRAVIOLET LIGHT OF SOME ANIONIC DETERGENTS (R_F 0·75)

Substance	Pinacryptol yellow		Pinacryptol yellow and rhodamine B	
	Before heating	After heating	Before heating	After heating
Alkylarylsulphonates	Orange	Orange	Orange	Orange
Alkyl sulphates	Orange	Pale yellow or blue	Orange	Blue
Sulphated alcohol ethoxylate*	Orange	Blue	Orange	Blue
Sulphated alkylphenol ethoxylate*	Violet	Violet	Orange	Orange and violet
Soaps	Pale yellow	None	Orange	Pale yellow

* The ethoxylates give positive tests with iodine and with cobalt thiocyanate.

Rhodamine B—The colours given with rhodamine B sprayed on top of pinacryptol yellow are in general similar to those given with pinacryptol yellow alone, but all anionic detergents and soaps now appear brilliant orange, and the spots due to non-ionic detergents are often more definite. If soaps and sulphated alkylphenol ethoxylates are known to be absent, a single spray of a mixture of equal volumes of the pinacryptol yellow and rhodamine B solutions may be used.

Similar changes in colour to those mentioned above occur when the paper is heated (see Table II).

Iodine—Dark brown spots are formed rapidly with polyethoxylates, amine oxides and cationic detergents. These spots last for several hours. Brown spots are also formed with alkanolamines, alkanolamides and metals, but they disappear in a few minutes when the paper is removed from the iodine vapour.

Spots due to polyethoxylates (both non-ionic and sulphated) appear dark green in ultraviolet light, whereas non-ethoxylated anionic detergents are unaffected by iodine (see Tables III and IV).

Bromocresol green—This gives blue spots with alkaline substances. Amine oxides (R_F 0·85) and cationic detergents (R_F 0·70) have already been detected. Triethanolamine (R_F 0·74), diethanolamine (R_F 0·57), monoethanolamine (R_F 0·43), monoisopropanolamine (R_F 0·50) and metals (R_F 0·00), which may have shown faintly before, now appear as prominent blue spots. Marker solution A will show the positions of tri-, di- and monoethanolamine (see Tables III and IV).

If anionic substances but no bases are found in a sample, ammonia, which does not show on the chromatogram, is probably present.

Cobalt thiocyanate—Bluish green spots are formed with polyethoxylates, amine oxides and cationic detergents. Diethanolamides also may give extremely faint colours.

The intensity of the colour with polyethoxylates depends on the degree of ethoxylation. Amine oxides and cationic detergents are distinguished from polyethoxylates because they give blue spots with bromocresol green (see Table III).

TABLE III

COLOUR REACTIONS OF NON-IONIC (R_F 0·90 TO 0·95) AND CATIONIC (R_F 0·70) SURFACE-ACTIVE AGENTS AND OF AMINE OXIDES (R_F 0·85)

Substance	PY or PY + RB	I	BCG	CT
Monoethanolamides	Pale blue or pink	Fades	None	None
Diethanolamides	Blue	Fades	None	Very weak
Ethoxylated monoethanolamides ..	Blue	Strong	None	Strong
Ethoxylated fatty alcohols	Blue	Strong	None	Strong
Ethoxylated alkylphenols	Blue or grey-violet	Strong	None	Strong
Quaternaries	Blue or pink	Strong	Blue	Strong
Amine oxides	Blue	Strong	Violet-blue	Strong

PY = Pinacryptol yellow (viewed in ultraviolet light).
PY + RB = Pinacryptol yellow and rhodamine B (viewed in ultraviolet light).
I = Iodine (brown spots).
BCG = Bromocresol green.
CT = Cobalt thiocyanate (blue-green spots).

The cobalt thiocyanate spray confirms the results of the iodine treatment, but, for unusual samples, one test may reveal spots that the other has missed.

p-*Dimethylaminobenzaldehyde*—This spray gives bright yellow spots with urea (R_F 0·31); the marker will show the position. The presence of urea usually indicates that a liquid detergent contains sodium alkylarylsulphonates, because urea is used to increase their solubility.

Some detergent sulphates are prepared by using sulphamic acid, and any traces of this in a sample will give yellow spots of low R_F value.

TABLE IV

COLOUR REACTIONS OF VARIOUS COMPONENTS OF COMMERCIAL DETERGENTS

Substance	R_F	PY or PY + RB	I	BCG	DMAB
Toluene sulphonates ..	0·25	Red	None	None	None
Xylene sulphonates ..	0·32	Yellow	None	None	None
Monoethanolamine ..	0·43	Pale grey	Brown (fades)	Blue	None
Diethanolamine ..	0·57	Pale grey	Brown (fades)	Blue	None
Triethanolamine ..	0·74	Pale grey	Brown (fades)	Blue	None
Isopropanolamine ..	0·50	Pale grey	Brown (fades)	Blue	None
Metals (Na, K, Mg) ..	0·00	Pale grey	Brown (fades)	Blue	None
Urea	0·31	None	None	None	Strong yellow

DMAB = *p*-Dimethylaminobenzaldehyde (other headings as in Table III).

PROCEDURE FOR SEPARATING METALLIC CATIONS—

In the general procedure all metallic cations stay at the origin. Usually only sodium and potassium are found in detergents, but occasionally magnesium is present. To distinguish between these metals, wash a sheet of Whatman No. 1 paper, and dry it as described in the previous procedure. Spot the paper with marker solution C and with the sample solutions. Dry the paper as before, and develop the chromatogram in the methanol solvent. Development takes only 2 to 2½ hours.

Dry the chromatogram at 80° C, spray with bromocresol green, and dry again at 80° C. Observe the blue spots. Sodium (R_F 0·41), potassium (R_F 0·19) and magnesium (R_F 0·00) are easily distinguished. The R_F value of sodium may be above 0·41 when potassium is present in the same sample.

Alkanolamines have high R_F values, and di- and triethanolamine are not so well separated as they are with the butanol solvent.

PROCEDURE FOR EXAMINING AMIDE HYDROLYSATES—

The methanol solvent is useful for separating hydroxy amines (mono- and diethanolamine and isopropanolamine), and aminosulphonic acids (taurine and *N*-methyl taurine) produced by hydrolysis of the corresponding amides. Hydrolyse the sample by boiling under reflux for 6 hours with 6 N hydrochloric acid. Remove fatty matter by extracting with light petroleum, and then evaporate the aqueous layer nearly to dryness. Add concentrated ammonia solution until the solution is alkaline, and apply spots to washed Whatman No. 1 paper. Use a spot of marker A. Develop for 2 to 2½ hours.

The spots may be revealed with bromocresol green, but a better method is to spray with a freshly prepared mixture of 20 ml of M sodium carbonate and 2 ml of acetaldehyde, and then with freshly prepared 1 per cent. sodium nitroprusside solution. Dry the paper at room temperature.

Primary amines (monoethanolamine R_F 0·63, isopropanolamine R_F 0·69 and taurine R_F 0·39) give khaki spots, whereas secondary amines (diethanolamine R_F 0·74 and *N*-methyl taurine R_F 0·55) give deep blue spots that slowly increase in intensity. The background is pink. If there is any doubt about the identification, leave the paper for at least half an hour before concluding the examination.

The test described below provides a useful and sensitive means of distinguishing between amines without chromatographic separation. Apply spots of the test solutions to filter-paper, and spray with the two reagent solutions. Primary amines give khaki spots, secondary give deep blue spots and tertiary give none.

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The Direct Spectrophotometric Determination of Antimony in Gold-Antimony Alloys and White Metals

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It has been found that 0·2 per cent. or more of antimony in gold-antimony alloys can be determined spectrophotometrically as the iodo-antimonite complex. In the method described the antimony colour is developed in a final solution of 25 ml, containing not more than 10 mg of alloy, without the need for separations. A calibration graph obtained from antimony solution only is also valid when gold is present. A correction, on the basis of 1 per cent. of gold being equivalent to 0·00035 per cent. of antimony, has to be deducted from the result by the proposed procedure, because of the slight colour due to gold.

Antimony contents greater than 1 per cent. in white metals can be determined by a similar procedure. An approximate knowledge of the tin content is, however, required to allow a correction for this element to be made (0·00653 per cent. of antimony is equivalent to 1 per cent. of tin).

AN alloy of nominal composition 0·8 per cent. of antimony and 99·2 per cent. of gold is used in transistor work for the production of ohmic contacts to n-type germanium base regions. The contacts are formed by heating the alloy to temperatures of more than 1500° C under high-vacuum conditions and depositing the vapour on the germanium surface. Since antimony is much more volatile than gold, it is necessary to consider whether the deposited alloy contains sufficient antimony to ensure an ohmic contact when applied to an n-type base. It is therefore necessary to determine antimony in films of alloy, often not more than 30 mg in weight, deposited on glass slides. It is also necessary to determine antimony in the alloy as supplied by the manufacturer, and, although there is generally no shortage of material, economy requires the use of a small weight of sample. As no suitable published method for determining antimony could be found, it became necessary to develop one.

The use of rhodamine B, which is a sensitive colorimetric reagent for antimony[1] and gold[2] was investigated. Experiments showed that, when Coppins and Price's method[1] was applied to solutions from which gold had been removed by precipitation with sulphurous acid, gold equivalent to about 0·1 per cent. of antimony was left behind to react with the rhodamine B. Accordingly, the use of this reagent was not pursued further.

Attention was then turned to the possibility of determining antimony as the yellow iodo-antimonite complex that is formed by adding potassium iodide and a reducing agent to an acid solution of antimony. The reducing agents proposed in the literature are sodium hypophosphite[3,4] and ascorbic acid.[5,6] Although these four papers discuss the behaviour of many metals in the reaction, they do not mention gold. It was therefore decided to investigate both modifications with the aim of finding conditions under which it would be possible to determine antimony in gold-antimony alloys without having to separate either antimony or gold, thus affording a rapid method of analysis.

The application of the iodo-antimonite reaction to the direct determination of more than 1 per cent. of antimony in white metals was also studied. There is a distinct need for a rapid method of determining antimony in small samples of a lead alloy containing about 10 per cent. of antimony, because n-type collector and emitter dots for some transistors are made of such alloys. Coppins and Price's method,[1] which is now part of a British Standard,[7] is only suitable for antimony contents of 1 per cent. or less.

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EXPERIMENTAL

GENERAL PROCEDURE—

Into a 25-ml calibrated flask were placed, by pipette, 5 ml of standard antimony solution equivalent to 0·100 mg of antimony; suitable volumes of 25 per cent. v/v sulphuric acid and a combined solution of potassium iodide and the reducing agent were then added to give the desired concentration. The contents of the flask were diluted to the mark with water, mixed, and the optical density of the solution was measured in a 4-cm cell against water at 426 mμ (the wavelength of maximum absorption in the visible region). A blank determination was made by omitting the standard antimony solution, and the blank value obtained was deducted from the original reading. When solutions of metals or alloys were used, they were added before the reagents.

COMPARISON OF THE REDUCING AGENTS—

With the reagent concentrations recommended by Rooney,[4] equivalent to 11·1 ml of 25 per cent. v/v sulphuric acid, 5 g of potassium iodide and 1 g of sodium hypophosphite in a final volume of 25 ml, there was a tendency for free iodine to form. This made necessary the addition of sodium thiosulphate solution. More serious was the fact that when gold was present it was precipitated and had to be removed by spinning in a centrifuge before the iodo - antimonite colour could be measured.

The conditions suggested by Elkind, Gayer and Boltz,[6] namely 7·5 ml of 25 per cent. v/v sulphuric acid, 1·75 g of potassium iodide and 0·125 g of ascorbic acid in a final volume of of 25 ml, were then examined. No free iodine could be detected with starch solution, and 10 mg of gold did not form a precipitate.

Hence sodium hypophosphite did not merit further study, and work was therefore directed towards finding the best conditions with ascorbic acid being used as the reducing agent.

OPTIMUM REAGENT CONCENTRATIONS—

McChesney[5] states that a reagent concentration equivalent to 8 ml of 25 per cent. v/v sulphuric acid, 2 g of potassium iodide and 0·25 g of ascorbic acid in a final volume of 25 ml gives a maximum optical density for the iodo - antimonite complex. As these concentrations differ from those used by Elkind, Gayer and Boltz,[6] a study was made to find optimum contions. In accordance with the results of McChesney[5] it was found that the optical density of the iodo - antimonite complex increased only slowly as more than 1·4 g of potassium iodide was taken. Ascorbic acid did not have much influence, provided that at least 0·075 g of it was present. The concentrations finally chosen, namely 8 ml of 25 per cent. v/v sulphuric acid, 2·5 g of potassium iodide and 0·25 g of ascorbic acid in 25 ml, were such that 0·100 mg of antimony gave an optical density of 0·640, which was slightly higher than when the concentrations suggested by McChesney or Elkind, Gayer and Boltz were used. Further, variations in the volume of 25 per cent. v/v sulphuric acid not exceeding ±1 ml could be ignored. The calibration graph of optical density against antimony content gave a straight line passing through the origin.

EFFECT OF GOLD—

Application of the selected reagent concentrations to solutions of pure gold showed that no precipitation occurred within 2 hours when 10 mg of gold were taken, although with 20 mg a slight precipitate was given. There was, however, a small optical density due to gold, directly proportional to its concentration. Ten milligrams gave an optical density of 0·023, which was equivalent to 0·00035 per cent. of antimony for 1 per cent. of gold. The calibration graph of optical density against antimony concentration obtained when 10 mg of pure gold were present was a straight line which, after correction for gold and the reagent blank value, passed through the origin and had the same slope as in the absence of gold. Hence, provided that a deduction on the above basis is made from the antimony result for a gold - antimony alloy, the use of pure gold in the construction of the calibration graph is unnecessary. As shown below, the amount of hydrochloric acid introduced when the alloy is dissolved in aqua regia is without effect on the colour of the iodo - antimonite complex.

APPLICATION OF THE METHOD TO WHITE METALS—

To establish whether or not the iodo - antimonite method could also be applied to white metals, experiments were carried out, with the slected reagent concentrations, on 5-ml aliquots taken after lead sulphate had been allowed to settle from the supernatant liquid of 100-ml solutions of 25 mg of either British Chemical Standard 177/1 or 178 in sulphuric acid. It was found that hydrochloric acid had to be present in the solution from which the aliquot was taken, in order to avoid retention of antimony by lead sulphate and consequent low recoveries. Four millilitres of 50 per cent. v/v hydrochloric acid, when added to the solution of the white metal before dilution to 100 ml, were sufficient to prevent any such loss.

As chlorides are stated to bleach the iodo - antimonite complex,[8] the effect of the equivalent of 0·2 ml of 50 per cent. v/v hydrochloric acid on the optical density of 0·100 mg of antimony was examined; no bleaching was observed. Hence the addition of 4 ml of 50 per cent. v/v hydrochloric acid to the solution of a white metal does not subsequently reduce the colour of the iodo - antimonite complex.

Optical-density measurements obtained with a corresponding solution of pure lead showed that this element was without effect. Similarly, as much as 0·5 mg of copper in the aliquot could be ignored.

Tin was found to form a pale yellow complex, the optical density of which increased rapidly as the wavelength of measurement was lowered, although there was no maximum above 390 mμ. The colour of this tin complex, unlike that of iodo - antimonite, was found to increase when the concentration of the sulphuric acid or the potassium iodide - ascorbic acid solution was raised; the colour was substantially reduced by even small amounts of hydrochloric acid. However, when reagent solutions were measured accurately, with pipettes, the optical density of the tin complex could always be reproduced. With 8 ml of 25 per cent. v/v sulphuric acid, 0·2 ml of 50 per cent. v/v hydrochloric acid, 2·5 g of potassium iodide and 0·25 g of ascorbic acid in a final volume of 25 ml, the optical density of the tin complex was directly proportional to the tin content of the solution. An optical density of 0·084 was given by 2 mg of tin, on the basis of which it was calculated that 1 per cent. of tin is equivalent to 0·00653 per cent. of antimony. Further, a calibration graph for antimony in the presence of as much as 2 mg of tin did not differ, after correction for the tin, from the one prepared from antimony only.

Hence, there is no need to add tin, lead or copper to the antimony solution when a calibration graph is being constructed for use in the analysis of white metals, and a correction need only be applied for the tin content.

METHOD

REAGENTS—

Use analytical-reagent grade chemicals whenever these are available.

Antimony solution A—Dissolve 0·3335 g of antimony potassium tartrate previously dried at 100° C and 0·25 g of tartaric acid in water, dilute the solution to 250 ml in a calibrated flask, and mix.

1 ml ≡ 0·500 mg of antimony.

Antimony solution B—By pipette place 10 ml of antimony solution A in a 250-ml calibrated flask, dilute to the mark with water, and mix.

1 ml ≡ 0·020 mg of antimony.

Sulphuric acid, 50 per cent. v/v—Prepare from sulphuric acid, sp.gr. 1·84.

Sulphuric acid, 25 per cent. v/v—Prepare from the 50 per cent. v/v sulphuric acid.

Hydrochloric acid, 50 per cent. v/v—Prepare from hydrochloric acid, sp.gr. 1·18.

Hydrochloric acid, 5 per cent. v/v—Prepare from the 50 per cent. v/v hydrochloric acid.

Nitric acid, 50 per cent. v/v—Prepare from nitric acid, sp.gr. 1·42.

Aqua regia, 50 per cent. v/v—Mix 3 volumes of 50 per cent. v/v hydrochloric acid and 1 volume of 50 per cent. v/v nitric acid. Discard the solution after 12 hours.

Potassium iodide - ascorbic acid solution—Dissolve 25 g of potassium iodide and 2·5 g of ascorbic acid in 41 ml of water. Store the solution in a brown glass bottle, and discard after 48 hours.

PREPARATION OF CALIBRATION GRAPH—

Place separately in six 25-ml calibrated flasks 0, 1, 2, 4, 6 or 8 ml of standard antimony solution B from a burette. Dilute the contents of each flask to 10 ml with water. Add, from pipettes, 8 ml of 25 per cent. v/v sulphuric acid and 5 ml of potassium iodide - ascorbic acid solution. Dilute to the mark with water, and mix. After 10 minutes, but within 2 hours, measure the optical density of the solution in a 4-cm cell against water at 426 mμ.

Deduct the optical density of the solution containing no added antimony from those of the remaining five. Plot these net values of optical density against the weights of antimony taken, and draw the best straight line through the origin to obtain the calibration graph.

PROCEDURE FOR GOLD - ANTIMONY ALLOYS CONTAINING 0·2 per cent. OR MORE OF ANTIMONY—

Weigh not more than 25 mg of sample containing not less than 0·05 mg or more than 0·38 mg of antimony. Place it in a 30-ml beaker, add, from a pipette, 1 ml of 50 per cent. v/v aqua regia, cover the beaker, and stand it on a boiling-water bath until the alloy has dissolved. Add to the contents of the beaker 5 ml of water and 50 mg of sulphamic acid, and boil gently for 2 minutes. Cool the solution to room temperature, add to it 10 ml of 50 per cent. v/v sulphuric acid, transfer to a 25-ml calibrated flask, dilute to the mark with water, and mix.

By pipette transfer a 10-ml aliquot to a 25-ml calibrated flask containing 8 ml of water. Add, from a pipette, 5 ml of potassium iodide - ascorbic acid solution, dilute to the mark with water, and mix. After 10 minutes, but within 2 hours, measure the optical density of the solution in a 4-cm cell against water at 426 mμ. Deduct the optical density of a reagent blank solution.

Refer the net optical density to the calibration graph and hence calculate the uncorrected antimony content of the alloy. Deduct from this figure the amount of antimony equivalent to the gold content, as described under "Experimental," and hence obtain the corrected antimony content of the alloy.

If it is desired to determine the value of the gold correction, weigh accurately about 100 mg of pure gold, and dissolve in 4 ml of 50 per cent. v/v aqua regia. Proceed as described above, and add 200 mg of sulphamic acid and 40 ml of 50 per cent. v/v sulphuric acid, and then dilute the solution to 100 ml in a calibrated flask. Transfer aliquots of 0, 2·5, 5·0, 7·5 and 10 ml to separate 25-ml calibrated flasks. Add to the contents of each flask sufficient 25 per cent. v/v sulphuric acid to give the equivalent of 8 ml, account being taken of the volume of 50 per cent. v/v sulphuric acid introduced with the aliquot. Dilute the contents of each flask to 18 ml with water, add 5 ml of potassium iodide - ascorbic acid solution as described under "Preparation of Calibration Graph," and continue as described to obtain a graph of optical density against weight of gold. From the slope of this graph calculate the amount of antimony equivalent to 1 part of gold.

PROCEDURE FOR WHITE METALS OF KNOWN TIN CONTENT CONTAINING MORE THAN 1 per cent. OF ANTIMONY—

Weigh not more than 50 mg of sample containing not less than 0·4 mg or more than 3 mg of antimony, or more than 40 mg of tin or 10 mg of copper. Place it in a 100-ml beaker, add 1 g of potassium hydrogen sulphate and 5 ml of sulphuric acid, sp.gr. 1·84, cover the beaker, and heat until the alloy has completely disintegrated. Avoid undue loss of acid by not allowing the solution to evolve fumes. Cool the solution, add to it 10 ml of water, and boil gently for 3 minutes. Cool the solution to room temperature, add from a pipette 4 ml of 50 per cent. v/v hydrochloric acid, transfer to a 100-ml calibrated flask, add 10 ml of 50 per cent. v/v sulphuric acid, dilute to the mark with water, and mix. Allow any lead sulphate to settle.

By pipette, transfer a 5-ml aliquot of the clear solution to a 25-ml calibrated flask. Add 6 ml of 25 per cent. v/v sulphuric acid from a pipette, and then 7 ml of water. Add from a pipette 5 ml of potassium iodide - ascorbic acid solution, and continue as described under "Procedure for Gold - Antimony Alloys" to obtain the uncorrected antimony content of the alloy. Deduct from this figure the amount of antimony equivalent to the tin content, as described under "Experimental," and hence obtain the corrected antimony content of the alloy.

If it is desired to determine the value of the tin correction, weigh accurately about 100 mg of pure tin, add 1 g of potassium hydrogen sulphate, and dissolve in 5 ml of sulphuric acid, sp.gr. 1·84. Proceed as described above, and add 10 ml of 50 per cent. v/v hydrochloric acid and 40 ml of 50 per cent. v/v sulphuric acid, and dilute the solution to 250 ml in a calibrated flask. Transfer aliquots of 0, 1, 2, 3, 4 and 5 ml to separate 25-ml calibrated flasks. Add to the contents of each flask sufficient 25 per cent. v/v sulphuric acid and 5 per cent. v/v hydrochloric acid, from pipettes, to give the equivalents of 8 ml and 2 ml, respectively, account being taken of the volumes of sulphuric acid and 50 per cent. v/v hydrochloric acid introduced with the aliquot. Dilute the contents of each flask to 18 ml with water, add 5 ml of potassium iodide - ascorbic acid solution as described under "Preparation of Calibration Graph," and continue as described to obtain a graph of optical density against weight of tin. From the slope of this graph calculate the amount of antimony equivalent to 1 part of tin.

Results

Table I shows typical results obtained by applying the proposed method to gold - antimony alloys and white metals.

Table I

Antimony contents of alloys

Alloy	Composition, %	Antimony content before correction, %	Correction, as antimony, %	Corrected antimony content, %
Gold - antimony	0·8 ± 0·1 of antimony	0·816	99 × 0·00035 = 0·035	0·78
Gold - antimony	0·9 ± 0·1 of antimony	0·943	99 × 0·00035 = 0·035	0·91
White metal (B.C.S. 177/1)	10·4 (10·30 to 10·50) of antimony 5·09 of tin 84·5 of lead	10·56	5 × 0·00653 = 0·033	10·5
White metal (B.C.S. 178)	7·51 (7·40 to 7·59) of antimony 84·0 of tin 4·08 of copper 3·86 of lead 0·40 of zinc	8·04	84 × 0·00653 = 0·549	7·49
Transistor dots, n-type	10·0 ± 0·1 of antimony	9·93	—	9·93

Discussion of results

The last column of Table I shows that, provided corrections for gold and tin are deducted according to their contents, which need only be known to within ±2 per cent., accurate results are obtained for antimony.

To apply the method to the analysis of gold - antimony alloys containing less than 0·2 per cent. of antimony, gold would have to be removed from a larger sample by precipitation as the element, and antimony determined on a suitable portion of the filtrate from which the sulphurous acid had been removed by boiling.[5]

As white metals generally contain only traces of bismuth, no provision has been made in the procedure for the positive interference of this element.[5] However, if more than 0·01 per cent. of it is present in an alloy, the deduction of an appropriate correction from the antimony result has to be considered. Under the conditions of the method 1 per cent. of bismuth is equivalent to 0·78 per cent. of antimony.

If the relative figures for the volumes of acids and potassium iodide - ascorbic acid solution as well as for the maximum weights of sample and elements, as stated in the method, are retained, it is permissible to vary the weights of alloy and the sizes of the aliquots and calibrated flasks used.

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SHORT PAPERS

A Substitute Reagent for Titanous Sulphate for Reducing Nitrate-N

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BREMNER and Shaw[1] have described a micro-diffusion method for determining ammonium-N and nitrate-N extracted from soils by shaking with potassium sulphate solution and then filtering off the suspension. In their method, titanous sulphate reduced nitrate-N to ammonium-N before distillation with magnesium oxide suspension to drive off the ammonia. Two series of micro-diffusion units were required, one without titanous sulphate for determining ammonium-N, the other with titanous sulphate for determining ammonium-N *plus* nitrate-N; a figure for nitrate-N was obtained by difference. Bremner[2] developed this method by using steam-distillation instead of micro-diffusion to drive off the ammonia, and he outlined the procedure described below. After the solution had been made alkaline with magnesium oxide, the ammonia was distilled into boric acid and titrated with standard acid; titanous sulphate was added to reduce nitrate-N to ammonium-N, which was then distilled as ammonia. By suitable pre-treatment of samples nitrite-N could also be determined. As technical-grade titanous sulphate is no longer available, a substitute reagent, involving the use of titanous chloride, has been developed.

In preliminary investigations I found that nitrate-N was not reduced quantitatively to ammonium-N either by titanous chloride or by "pure" titanous sulphate. (The sulphate was prepared from titanous chloride by adding sulphuric acid, evaporating to dryness in a vacuum desiccator over calcium chloride and sodium hydroxide, and dissolving the resulting mass in water to give the original volume of titanous chloride). However, addition of ferrous sulphate to the titanous chloride or sulphate solutions greatly increased the percentage of nitrate-N recovered as ammonia when standard solutions were distilled.

When standard solutions of nitrate-N were distilled with reagents prepared from titanous chloride and titanous sulphate solutions containing different amounts of added ferrous sulphate, nitrate-N was recovered quantitatively over a limited range of ferrous ion concentrations. Reagents prepared from titanous chloride *plus* ferrous sulphate reduced nitrate-N quantitatively in the range 0 to 300 µg of nitrogen, whereas those from titanous sulphate *plus* ferrous sulphate quantitatively reduced at least 500 µg of nitrate-N. To investigate the effects of chloride and of sulphate on the reduction of nitrate-N, standard solutions of nitrate-N were prepared in N potassium sulphate and N potassium chloride (corresponding with soil extracts), and reagents were prepared from technical titanous sulphate solution, titanous chloride solution *plus* ferrous sulphate and titanous chloride solution *plus* ferrous chloride. Both standard solutions were distilled with each reagent. Five-hundred micrograms of nitrate-N were recovered quantitatively as ammonia from the mixtures containing only sulphate as anion; least nitrate-N was recovered from solutions containing only chloride. In a satisfactory reagent the titanous chloride must therefore be converted to sulphate and the correct amount of ferrous sulphate must be added. A simpler and quicker procedure for making the reagent is to add iron powder to titanous chloride solution acidified with sulphuric acid, and while the iron is dissolving to boil the mixture to expel as much hydrochloric acid as possible. The effects of different amounts of sulphuric acid and of iron added to the reaction mixture were investigated. Nitrate-N was reduced quantitatively over a limited range of ferrous concentrations. The amount of acid was critical; with a small excess over that theoretically required to replace chloride by sulphate, nitrate-N was not recovered quantitatively, and with too large an excess much solid material was deposited from the solution on standing. A satisfactory reagent is prepared by the procedure described below.

METHOD

REAGENTS—

Titanous chloride—An approximately 15 per cent. w/v solution of the technical-grade material.

Iron powder—Reduced by hydrogen (also called "reduced iron").

Sulphuric acid, sp.gr. 1·84.

PROCEDURE—

Add 10 ml of sulphuric acid to 100 ml of titanous chloride solution in a 250-ml conical flask, mix, and set aside for 5 to 10 minutes. Weigh 1·5 g of iron powder, and add about 1 g to the contents of the flask. Rapidly heat the mixture to boiling, and then adjust the heat input to allow the iron to dissolve as quickly as possible. When the vigorous reaction has subsided, boil for 1 minute more, and then cool slightly. Add the remaining iron, heat again, and boil until the vigorous reaction begins to subside. Remove the source of heat, and allow the mixture to cool. When cool, dilute the reagent to 100 ml with water, and store in a stoppered dark-coloured bottle.

For use, dilute the reagent with an equal volume of water, and use 2 ml for each determination. This should quantitatively reduce up to 500 μg of nitrate-N. Check the total reducing capacity by adding 2 ml of dilute reagent to 2000 μg of nitrate-N and distilling the ammonia formed into boric acid. Between 1200 and 1400 μg of nitrate-N should be reduced. The undiluted reagent keeps for at least 6 weeks; it should be diluted daily as required.

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A Volumetric Method for determining Phosphate and Arsenate in a Mixture

BY J. BASSETT

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THE indirect complexometric determinations of phosphate[1] and arsenate[2] with standard bismuth nitrate solution have been reported. The method described here makes use of this type of procedure for determining the total phosphate and arsenate in a mixture, the arsenate being separately determined iodimetrically.[3,4,5]

EXPERIMENTAL

OPTIMUM CONCENTRATION OF NITRIC ACID TO ALLOW PRECIPITATION OF BISMUTH PHOSPHATE AND ARSENATE—

Ross and Hahn[6] have shown that bismuth phosphate is quantitatively precipitated from solutions by concentrations of nitric acid as high as 0·65 N. Preliminary experiments in which a complexometric procedure similar to that described under "Method" was used indicated that the optimum concentration of nitric acid for quantitatively precipitating bismuth arsenate was 0·50 to 0·60 N.

INVESTIGATION OF INTERFERENCES—

The interference of foreign ions in the complexometric determination of total phosphate and arsenate was studied. The results showed that the ions Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Al^{3+} and Cr^{3+} do not interfere when present at a molar concentration approximating to that of the total phosphate and arsenate present. The ions Pb^{2+}, Fe^{3+}, Cl^{-} and SO_4^{2-} interfere.

METHOD

REAGENTS—

Bismuth nitrate solution, 0·02 M *in* 0·9 N *nitric acid*—Prepare from pure bismuth oxide, and standardise by titration against standard ethylenediaminetetra-acetate solution with xylenol orange as indicator.[7]

Standard disodium ethylenediaminetetra-acetate (*EDTA*) *solution,* 0·01 M.

Standard sodium thiosulphate, 0·05 M.

Xylenol orange indicator solution, 0·5 *per cent. w/v, aqueous.*

Potassium iodide—Analytical-reagent grade.

Sodium hydrogen carbonate—Analytical-reagent grade.

Amberlite IR–120 (*H*) *cation-exchange resin*—Analytical grade.

REMOVAL OF INTERFERING CATIONS—

Prepare a column (250 mm × 10 mm) of Amberlite IR–120 cation-exchange resin in the hydrogen form, and wash out any excess of acid by passing 100 ml of water through the resin. Dissolve the sample, containing about 5 millimoles each of phosphate and arsenate, in 6 ml of 8 N nitric acid, and dilute to 50 ml with water. Allow the resulting solution, containing not more than about 10 millimoles of interfering cations, to flow through the column at a rate of 3 to 4 ml per minute. Wash the beaker that contained the mixed phosphate and arsenate solution with four 25-ml portions of water, and allow these washings to flow through the resin column. Collect the total effluent in a 500-ml calibrated flask, and make up to the mark with water.

COMPLEXOMETRIC DETERMINATION OF TOTAL PHOSPHATE AND ARSENATE—

By pipette, place 25 ml of the cation-free phosphate and arsenate solution in a beaker. Heat the solution to about 90° C, and, if sulphate is present, add 10 ml of a hot 10 per cent. solution of barium nitrate in 0·5 N nitric acid. Remove chloride, if present, by adding 10 ml of a hot 10 per cent. solution of silver nitrate, also in 0·5 N nitric acid. Add, with constant stirring, 35 ml of the bismuth nitrate solution from a burette. Boil the solution, set it aside for 15 minutes, and filter through a G4 sintered-glass crucible. Wash the precipitate, by decantation, with six 10-ml portions of hot 0·1 N nitric acid. Adjust the nitric acid concentration of the combined filtrate and washings to 0·1 to 0·2 N, add a few drops of xylenol orange indicator, and titrate with the standard EDTA solution until the colour changes from red to lemon-yellow. Calculate the total phosphate and arsenate content from the equation—

$$\text{Phosphate } \textit{plus} \text{ arsenate, millimoles} = (X - Y) \times 20,$$

where X is the number of millimoles of Bi^{3+} originally added and Y is the number of millimoles of Bi^{3+} present in the filtrate and washings.

IODIMETRIC DETERMINATION OF ARSENATE—

By pipette, place 50 ml of the cation-free phosphate and arsenate solution in a glass-stoppered 250-ml conical flask. Add 35 ml of concentrated hydrochloric acid, cool the solution thoroughly, and add two 0·5-g portions of analytical-reagent grade sodium hydrogen carbonate and then a 1-g portion of analytical-reagent grade potassium iodide. Insert the stopper in the flask, swirl the solution to dissolve the potassium iodide, and set aside for 5 minutes. Titrate rapidly and with constant swirling of the solution against the standard sodium thiosulphate solution to the disappearance of the last trace of yellow colour; view against a white background. Carry out a blank titration with 50 ml of water instead of the phosphate and arsenate solution, and subtract the blank titre from the first sodium thiosulphate titre to obtain the corrected sodium thiosulphate titre. Calculate the arsenate content of the sample from the equation—

$$\text{Arsenate, millimoles} = 5\ (T \times \mathrm{N})$$

where T is the corrected sodium thiosulphate titre and N the normality of the sodium thiosulphate solution. Subtract the arsenate content from the total phosphate and arsenate content to obtain the amount of phosphate (in millimoles) present in the sample. If required, convert these values to milligrams of arsenate and of phosphate—

$$\text{Arsenate content, mg} = \text{Arsenate content, millimoles} \times 138{\cdot}91$$
$$\text{Phosphate content, mg} = \text{Phosphate content, millimoles} \times 94{\cdot}97$$

RESULTS

The results in Table I were obtained with mixed phosphate and arsenate solutions each containing about 10 millimoles of foreign ion.

TABLE I

RESULTS

Foreign ion added	Arsenate taken, millimoles	Phosphate taken, millimoles	Total phosphate + arsenate found, millimoles	Arsenate found, millimoles	Phosphate found (by difference), millimoles
Cu^{2+}, Pb^{2+}	5·20	5·05	10·20	5·20	5·00
Cu^{2+}, Pb^{2+}, Fe^{3+} ..	5·02	4·91	9·95	5·03	4·92
SO_4^{2-}	5·10	5·11	10·17	5·11	5·06
Cl^-	5·13	5·05	10·23	5·15	5·08

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A Rapid Test for Anionic Detergents in Drinking Water

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THE current general use of anionic synthetic detergents, with their consequent constant presence in domestic sewage, renders such material an excellent indicator of the possible pollution of a drinking water. This is particularly so for private shallow wells in places associated with cess-pit or septic tank disposal of sewage.

It has been shown[1] that the absence of coliform organisms from a water is not necessarily an indication of the absence of intestinal viruses. The poliomyelitis and coxsackie viruses, for example, are considerably smaller than the coliform bacteria, and their ease of movement through the soil is correspondingly greater. The ions of the common synthetic detergents are smaller still and therefore even less affected by the filtering action of the soil,[2] although adsorption is likely to occur to some extent. Consequently the appearance of anion-active material in a previously pure well water may be a preliminary indication of potential viral pollution, long before any evidence of coliform organisms is obtained.

EXPERIMENTAL

Several dyestuffs have been used for the colorimetric determination of anion-active matter in water and sewage. The most used material is methylene blue,[3,4,5,6] but this is unsuitable for a simple detection test because of the different amounts of the several interfering impurities usually present[6] in commercial samples. These can be extracted from an alkaline solution of the dye, but the resulting reagent is unstable.

Toluidine blue-O has recently been used[7] in a field test for detergents in well water. A range of colours prepared from copper sulphate and cobalt chloride solutions is used as a standard. The lowest standard quoted is for 0·1 mg per litre and the blank standard solution contains cobalt chloride, indicating that the dyestuff is chloroform-soluble to some extent under the conditions of the test. It has also been found that toluidine blue-O contains a chloroform-soluble impurity.

Methyl green has been suggested[8] as a reagent for the determination of anionic detergents in sewage. This method has not been widely used because of difficulties inherent in the choice of benzene as the extracting solvent. The authors found that the dye was soluble in chloroform, but it is apparent that they had overlooked the presence of crystal violet (methyl violet) as a common[9] impurity in methyl green. Gould[10] has found that the absorption of the dye - surface active agent complex produced by different samples of methyl green can vary significantly. This is due to the presence of various small amounts of crystal violet.

Crystal violet is insoluble in benzene, but is readily removed from a neutral aqueous solution of methyl green by extraction with chloroform. The use of a solution purified in this way leads to no coloration of the chloroform when detergent-free water is examined by the method proposed below.

The proposed procedure will detect with confidence 1 μg of anion-active material, as Manoxol O.T. (di(2-ethylhexyl) sodium-sulphosuccinate), corresponding to a concentration of 0·005 mg per litre. Ions commonly present in drinking water, *e.g.*, nitrate, chloride, sulphate and phosphate, do not interfere, and urine does not give a positive response to this test. Both alkylsulphates and alkylbenzenesulphonates are detected by this method, but soap gives no colour. An estimate of the amount of anion-active matter present can be obtained by comparing the depth of colour given by a sample with a series of suitable standards similarly treated at the same time. In common with other methods involving extraction from acid solution, quantitative recovery of anion-active material may not be achieved in the presence of protein.[4]

The test described below is designed for field use and can be completed in under 5 minutes. Waters showing a positive reaction may then be examined quantitatively, preferably by an infrared method[11] whereby positive identification of the active matter can also be achieved.

METHOD

All glassware used, both for the preparation and storage of reagents and during the analysis, should be freed from adsorbed surface-active material by washing with diluted nitric acid (1 + 1) and then thorough rinsing with de-ionised water.

REAGENTS—

All materials should be of recognized analytical quality. De-ionised water should be used throughout.

Hydrochloric acid, approximately 2 N.

Chloroform.

Methyl green solution—Dissolve 0·01 g of methyl green (C.I. No. 42585) in water, and dilute to 500 ml. Extract sufficient of this solution for each day's use with 10-ml portions of chloroform until a colourless extract is obtained, usually four times. Filter to remove droplets of chloroform.

PROCEDURE—

To 200 ml of the sample contained in a glass-stoppered 250-ml separating funnel add 5 ml of 2 N hydrochloric acid and 5 ml of extracted methyl green solution. Add 5·0 ml of chloroform, shake vigorously for 30 seconds, and allow the layers to separate. Compare the colour of the solvent layer with that given by similar treatment of 200 ml of de-ionised water. A green colour indicates the presence of anion-active material in the sample.

RESULTS

Some drinking waters from different sources have been examined by the proposed method and by a methylene blue method.[6] The results are shown in Table I.

TABLE I

RESULTS

Source of water	Total hardness, as $CaCO_3$, mg per litre	Anion-active material found— by proposed method	by methylene blue method,[6] as Manoxol O.T., mg per litre
River 1*	100	Present	0·005
River 2	260	Present	0·07
River 3	15	Trace	0·001
Stream	15	Present	0·005
Spring 1	215	Absent	Nil
Spring 2	245	Present	0·005
Borehole 1	90	Absent	Nil
Borehole 2	140	Absent	Nil
Well	120	Absent	Nil
Distilled	—	Present	0·003

* This water was coloured, Hazen 70.

Permission to publish this paper has been given by the Government Chemist, Department of Scientific and Industrial Research.

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The Determination of Maneb in the Presence of Copper

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THE usual methods for determining dithiocarbamates in fungicides depend on decomposition by acid to give carbon disulphide. The latter is converted to xanthate by absorption in ethanolic potassium hydroxide, and the resulting xanthate is titrated with iodine under controlled conditions.

Before Roth[1] proposed his pyridine - formic acid method for determining maneb (manganese ethylene-1,2-bisdithiocarbamate), the principal methods used were those of Rosenthal, Carlsen and Stanley[2] and of Clarke *et al.*[3] Modifications to Clarke's method have been introduced[4,5] to try to overcome the inherent unwettability by sulphuric acid of maneb. In all the methods reported in the above-mentioned literature, copper interferes with the determination. Rosenthal, Carlsen and Stanley suggested the addition of sodium sulphide to reduce this interference. With commercial fungicide formulations containing maneb (or zineb) and a copper fungicide we have been unable to obtain satisfactory results with sodium sulphide solution. This is probably because it is usually extremely difficult to wet the sample completely with sodium sulphide solution before the acid is added to decompose the dithiocarbamate. Surface-active agents gave no improvement.

A fairly effective but rather cumbersome method for overcoming interference by copper, at least partly, was found in the use of zinc sulphide. The powdered sample of copper - maneb (zineb) formulation was intimately mixed with finely divided zinc sulphide, care being taken to avoid loss of material. The mixture was then transferred to the reaction flask and the decomposition, evolution of carbon disulphide and titration were carried out in the usual way.

Provided that the zinc sulphide is finely divided, results obtained when phosphoric acid is used for decomposition[2] are similar to those obtained in the absence of copper. It is essential to allow 90 minutes for evolution of carbon disulphide in the presence of copper and zinc sulphide. The results tabulated below show the effect of zinc sulphide on the determination of maneb in 0·3 g of technical maneb in the absence of copper. (The figure of 76·5 per cent., obtained in the absence of zinc sulphide, was taken as the "true" value.)

Weight of zinc sulphide, g	0·0	0·0	0·45	0·48	0·45	0·45	0·90	0·86
Maneb found in 0·3 g of technical maneb, %	76·5	76·5	77·0	78·5	78·5	77·0	77·5	78·5

Table I shows the effect of zinc sulphide on the determination of maneb in technical maneb to which had been added copper oxychloride equivalent to 0·6 g of copper. In the absence of zinc sulphide, results of from 10 to 35 per cent. were obtained.

TABLE I

EFFECT OF ZINC SULPHIDE IN THE PRESENCE OF COPPER ON THE DETERMINATION OF MANEB

0·6 g of copper was added

Weight of technical maneb, g	Theoretical excess of zinc sulphide, %	Maneb found, %
0·45	50	76·0
	100	76·0
0·30	10	72.5, 73.5, 73·0
	50	75·0, 74·5, 75·5, 75·5
	100	77·0, 76·5, 76·0
0·16	50	76·5, 78·0
	100	77·0, 76·5

From the results it will be seen that zinc sulphide affected the determination even in the absence of copper. This is not thought to be due to hydrogen sulphide escaping the absorption traps in the apparatus, since these had previously been shown to be highly efficient.

The results obtained in the presence of copper probably depend very much on the intimacy of the mixing of sample with zinc sulphide and the mode of addition of the acid.

At the time when this work was done (1960) the effect of ethylene thiuram monosulphide and ethylene thiourea on the determination of maneb was also briefly investigated. Both compounds interfere seriously in the phosphoric acid method. In the presence of ethylene thiourea, the presence of zinc sulphide with or without copper did not significantly affect the interference quantitatively. With ethylene thiuram monosulphide, the presence of zinc sulphide slightly increased the interference and zinc sulphide plus copper approximately doubled it.

It is known that Clarke's method (in which dilute sulphuric acid is used) is not so seriously affected by ethylene thiourea and ethylene thiuram monosulphide. The use of zinc sulphide to avoid interference by copper in Clarke's method has not been investigated because of early difficulties in obtaining quantitative results with maneb only.

The proposed procedure cannot be regarded as particularly accurate, since it depends on several factors that are difficult to control. It does, however, serve the purpose of determining maneb in the presence of a large excess of copper and no other methods have been noted in the literature that give reasonably satisfactory results.

METHOD

Accurately weigh sufficient sample to contain about 0·3 g of maneb into a clean glass tube 1½ inches long and ½ inch in diameter. If copper is present, weigh about 1·5 times its stoicheiometric equivalent of pure grade zinc sulphide (previously finely ground in an agate mortar) into the tube. Add 2 ml of water to the contents of the tube, and mix to a uniform "cream" with a 3-mm glass rod. Wash the rod with a further 2 ml of water, and add the washings to the contents of the tube. Transfer the tube and its contents to the reaction flask, and continue the determination of carbon disulphide by using phosphoric acid as in Rosenthal's method.

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A Modified Kipp's Apparatus Suitable for the Electrolytic Generation of Oxygen

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BY suitable modification, including the provision of two platinum-gauze electrodes, an ordinary one-litre Kipp's apparatus can be converted to a simple and robust electrolytic gas generator. I am not aware of any previous description of this application of the Kipp's apparatus or of its use in any commercially available electrolytic gas generator. An apparatus[1] described many years ago appears to function as a Kipp's apparatus, but differs from it in many respects. In daily use over a period of several months the apparatus shown in Fig. 1 has proved to be convenient, reliable and safe in operation.

The electrodes are constructed from 40-mesh platinum gauze and each has an area of about 4 sq. cm. The cathode, A, is attached to a short platinum wire, 0·5 mm in diameter, welded to a tungsten wire 24 cm long and 1·0 mm in diameter. The welded junction and a few millimetres of the adjacent wires are embedded in Pyrex-glass at the lower end of the 7-mm bore tube, B. The remaining length of tungsten wire passes through the constriction at the top of this tube and is there secured by a suitable cement. The tube, C, which has an internal diameter of 15 mm, is expanded to 25 mm at the lower end over a length of about 25 mm. It serves to minimise contact between the bulk of the electrolyte in bulb D and the hydrogen liberated at the electrode. The anode in the lowest bulb is attached to a 0·5-mm diameter platinum wire welded at E to a 15-cm length of 1·0-mm diameter tungsten wire or 20 s.w.g. copper wire. The platinum wire beyond the weld is embedded in Pyrex-glass as shown at F in Fig. 1, and the free end of the tungsten or copper wire is secured by cement in the constricted end of the hollow ground-glass stopper, G.

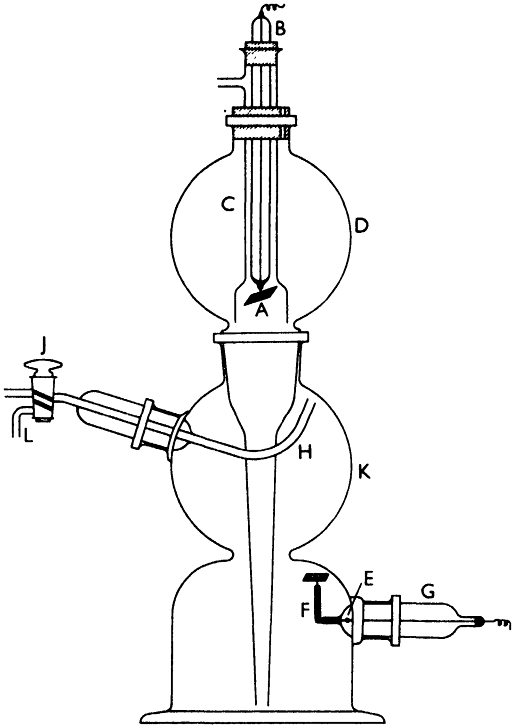

Fig. 1. Electrolytic oxygen generator

The 4-mm bore tube, H, sealed to tap J, terminates just below the top of the central bulb, K. It facilitates both the removal of air from this bulb and the use of the maximum capacity of the bulb. The generator is prepared for use by removing the electrode assembly A, B and C, and, with tap J open, 1·0 M sulphuric acid is poured into top bulb D until it has risen in the central bulb to just below the top of tube H. Tap J is closed, and the addition of electrolyte is continued until the depth of liquid in the top bulb is estimated to be sufficient to cover electrode A. The electrode assembly is replaced, the generator is connected to the mains a.c. supply through a suitable rectifier and Variac autotransformer, and the current is adjusted to about 0·4 amp, corresponding to 1·4 ml of oxygen per minute. When central bulb K contains about 500 ml of oxygen, tap J is opened, and the gas is expelled through tube L. The tap should be opened cautiously at first, until any electrolyte present in tube H has been driven out.

This procedure is repeated once more, and the electrolysis is then continued until bulb K is completely filled with oxygen. The apparatus is now ready for use. Since the current is automatically switched off when the level of liquid in the lowest bulb falls below the electrode, the generator can be operated overnight without attention. With the electrical components used (Variac type AS–80–31 and four metal rectifiers) no arcing occurs at the anode when the circuit is broken.

If more complete removal of air from the oxygen is desired, a few pieces of Drikold should be added to the electrolyte before the generator is filled. The removal of air from the uncharged generator can be effected in the same manner. If the presence of carbon dioxide in the oxygen cannot be tolerated, it can easily be removed by passing the gas over Carbosorb or any other suitable absorbent.

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Book Reviews

PRACTICAL PHARMACEUTICAL CHEMISTRY: QUANTITATIVE ANALYSIS. By A. H. BECKETT, D.Sc., Ph.D., F.P.S., F.R.I.C., and J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C. Pp. viii + 378. London: The Athlone Press. 1962. Price 63s.

This publication is intended primarily as a practical training guide on quantitative analysis for students preparing for a degree in pharmacy or similar qualification. It should save the authors and others similarly placed, such as in overseas schools of pharmacy, a lot of time from preparing practical instruction schedules. It is also hoped to serve as a work of reference when training has been completed.

The authors have taken the student methodically through the methods used for official drugs, although brief mention is also made of a few newer techniques.

The opening chapter on the principles of the quantitative tests for impurities seems to have been put in merely for completeness. After this, however, the subject is treated systematically in chapters giving first a brief outline of the technique and theoretical basis of quantitative analysis, and then giving accounts of various volumetric methods, gravimetric analysis, solvent extractions, analytical applications of emission and absorption techniques and polarography.

It is inevitable in a treatise designed to cover the period from initiation into analytical methods to the use of more complex techniques covering the examination of drugs and medicinal chemicals that the treatment should be out of balance. This would have been less apparent if the detailed steps in simple calculations were not repeated for all experimental exercises—for a student capable of assimilating the more abstruse parts of the work, a few examples would be all that were necessary.

The authors in giving such scant attention to the now well established modern techniques that have already superseded official methods in more advanced laboratories and concentrating on a syllabus to cover the methods officially applied to drugs cannot expect to have written a text-book of modern drug analysis. In fairness, they do not make this claim, and in providing a practical training guide to pharmacy students they have more than succeeded in their task and have taken the student well into the understanding of pharmaceutical analysis and the basic principles of official methods.

D. C. GARRATT

POISONING BY DRUGS AND CHEMICALS: AN INDEX OF TOXIC EFFECTS AND THEIR TREATMENT. By PETER COOPER, F.P.S. Second Edition. Pp. x + 264. London: Alchemist Publications. 1962. Price 25s.

This would be a useful book to keep in any chemical laboratory. It is arranged in alphabetical sequence and contains references to most of the toxic chemicals likely to be encountered.

Each substance is described under Synonyms, Occurrence, Pharmacological Action, Absorption and Excretion, Toxic Reactions, Suggested Treatment and Aids to Identification. Should a member of a laboratory accidentally imbibe a chemical substance, this is the book to fly to for guidance on how to act.

R. F. MILTON

NAMING ORGANIC COMPOUNDS: A GUIDE TO THE NOMENCLATURE USED IN ORGANIC CHEMISTRY. By E. H. TINLEY, B.Sc., F.P.S. Pp. vi + 48. London: Alchemist Publications. 1962. Price 12s. 6d.

A well known authority has written "organic chemical nomenclature is an infuriating subject." It is not surprising, therefore, that the ten chapters covering 36 pages and 9 pages of appendixes between the covers of this slim volume do not probe very deeply.

The chapters on aliphatic, homocyclic and heterocyclic compounds, steroids and carbohydrates average 4 pages apiece and those on optical activity and geometrical isomerism 3 pages each. The last two deal rather more with descriptions of the phenomena than with nomenclature. The examples cited throughout the book have a marked pharmaceutical flavour and in this connection it is of interest to record that in the November 1962 booklet of "Approved Names," at least half of the compounds listed are heterocyclic compounds; the 4-page chapter dealing with such chemicals might usefully have been expanded, possibly at the expense of that on the D.T.P. system, which is "not intended to replace the common names which are now used for simple ring compounds."

A chapter on Pronunciation is unusual and interesting. It does not, however, get as far as to indicate whether for names such as leucopterin and amethopterin, the "p" is silent and, if not, whether it should be linked with the preceding or succeeding letters; thebacon would also be a nice morsel here.

There are several errors, *e.g.*, diphenyl for biphenyl (p. 42); *cyclo*propane for cyclopropane, etc. (pp. 44 and 45); oestrone for oestrane (twice, p. 45); hexanedianoic acid for hexanedioic acid acid (p. 8) and the phrase "a glyoxaline (formerly called an iminazole) ring" (p. 16; p. 41) would run more accurately as "an imidazole (formerly called a glyoxaline or an iminazole) ring." Ethisterone is undesirably named on the "-ol-one" system on p. 38 and more correctly as "-hydroxy - - - one" on p. 23, though "-androst-4-en-3-one" would be preferred in British nomenclature.

B. A. ELLIS

ROCK-FORMING MINERALS. Volume 5. NON-SILICATES. By W. A. DEER, M.Sc., Ph.D., F.R.S., R. A. HOWIE, M.A., Ph.D., F.G.S., and J. ZUSSMAN, M.A., Ph.D., F.Inst.P. Pp. x + 371. London: Longmans, Green and Co. Ltd. 1962. Price 95s.

In the fifth volume of this work the structure, chemistry (including analyses), optical and physical properties, distinguishing features and the paragenesis of 51 important non-silicate minerals are described and discussed. These are grouped as oxides, hydroxides, sulphides, sulphates, carbonates, phosphates and halides and, to mention only a few of them, include cassiterite, corundum, ilmenite, perovskite, spinel, chromite, brucite, limonite, pyrite, galena, gypsum, dolomite, apatite, monazite and fluorite.

Dealing as it does with minerals of more varied types, this volume may appeal to a wider range of analysts than do the other volumes of this book (see *Analyst*, 1962, **87**, 607 and 914). The mineral analyst, in particular, will find a great deal of interest to him, and many references for further reading.

L. S. THEOBALD

HANDBUCH DER KOLORIMETRIE. Band I. KOLORIMETRIE IN DER PHARMAZIE. By Dr. rer. nat. BOHUMIL KAKÁČ and Dipl.-Ing. ZDENEK J. VEJDĚLEK. Translated by Dipl.-Ing. E. HACHOVÁ. Pp. xvi + 1139 + 12 plates. Jena: Veb Gustav Fischer Verlag. 1962. Price DM 79.20.

This volume embraces detailed accounts of the colorimetric determination of alkaloids, glycosides and aglycones, plant substances other than alkaloids and glycosides, vitamins and antibiotics. The whole work comprises three volumes, the second covering synthetic drugs, steroids, hormones and inorganic acids and the third the colorimetric assessment of substances of biological significance. A translation into German from the original Czech language is promised for all three volumes, thus rendering this exceptionally fine treatise readily available to all chemists.

The thoroughness with which the subject has been covered is outstanding, all preliminary manipulations of crude drugs and their various pharmaceutical preparations being carefully described and, it would seem, every reference to the relevant scientific literature from the first up to the year 1959 being quoted, the span sometimes extending to a full century. In illustration of the exhaustive treatment, it may be noted that the section on alkaloids occupies 274 pages; under morphine alone there are 140 references, and the account of the colorimetric estimation of the alkaloids of ergot occupies 23 pages. The same thorough treatment is accorded to the glycosides and, as an example, the relative value of the various proposals for the colorimetric assessment of the digitalis group is carefully discussed and full details of all the methods are given. To this sub-section there are 212 references and, as far as the reviewer can ascertain, nothing significant has been omitted.

A feature of noteworthy interest is the account given of the colorimetric determinations of upwards of 40 plant substances other than alkaloids and glycosides, including menthol, rotenone and capsaicin. The vitamins are dealt with in 218 pages and examples of chromatographic separations are included. The final section is devoted to antibiotics, which, although a limited field so far as visual colorimetric analysis is concerned, makes for completeness. The indexing and general production are all that can be desired and the work is to be highly commended to those concerned with this field of analytical chemistry.

NOEL L. ALLPORT

Publications Received

MODERN METHODS OF PLANT ANALYSIS. Volume V. Founded by K. PAECH and M. V. TRACEY. Continued by H. F. LINSKENS and M. V. TRACEY. Pp. xxvii + 535. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 98.

GAS CHROMATOGRAPHY. By HOWARD PURNELL. Pp. viii + 441. New York and London: John Wiley & Sons Inc. 1962. Price 90s.

SALT-WATER PURIFICATION. By K. S. SPIEGLER. Pp. x + 167. New York and London: John Wiley & Sons Inc. 1962. Price 57s.

CHROMATOGRAPHY AND ELECTROPHORESIS ON PAPER: A TEACHING LEVEL MANUAL. By J. G. FEINBERG, B.Sc., M.Sc., D.V.M., M.I.Biol., and IVOR SMITH, B.Sc., Ph.D., F.R.I.C., M.I.Biol. Pp. xii + 130. London: Shandon Scientific Co. Ltd. 1962. Price 21s.

PROGRESS IN NUCLEAR ENERGY. Series IX. ANALYTICAL CHEMISTRY. Volume 3. Parts 1–3. Edited by CARL E. CROUTHAMEL. Pp. vi + 93. Oxford, London, New York and Paris: Pergamon Press. 1962. Price 30s.

AZEOTROPIC DATA-II. Compiled by LEE H. HORSLEY and WILLIAM S. TAMPLIN. Pp. vi + 100. Washington, D.C.: American Chemical Society. 1962. Price $4.50.
Advances in Chemistry Series No. 35.

AN INTRODUCTION TO THE THEORY OF FLOTATION. By V. I. KLASSEN, Dr. Tech. Sc., and V. A. MOKROUSOV, Cand. Tech. Sc. Translated by J. LEJA, B.Sc., Dipl. Ing., Ph.D., and G. W. POLING, B.Sc., M.Sc. Pp. xiv + 493. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 126s.

GAS CHROMATOGRAPHY 1962. Edited by M. VAN SWAAY. Pp. lii + 411. London: Butterworth & Co. (Publishers) Ltd. 1962. Price 100s.
Proceedings of the fourth symposium organized by the Fachgruppe Analytische Chemie of the Gesellschaft Deutscher Chemiker and the Gas Chromatography Discussion Group of the Hydrocarbon Research Group of the Institute of Petroleum, held at the Auditorium Maximum, Hamburg 13–16 *June*, 1962.

BODENPHYSIK UND BODENKOLLOIDIK. By Professor Dr. JÁNOS DI GLERIA, Dr. ANDOR KLIMES-SZMIK and Dr. MIKLÓS DVORACSEK. Pp. 795. Budapest: Akadémiai Kiadó. 1962. Price $19.50.

QUALITATIVE ANION - CATION ANALYSIS: AN INTERPRETATIVE LABORATORY TEXT OF SEMIMICRO PROCEDURE IN BASIC COLLEGE CHEMISTRY. By EMIL J. MARGOLIS. Pp. x + 300. New York and London: John Wiley & Sons Inc. 1962. Price 38s.

Reprints of "Classification of Methods for Determining Particle Size"

REPRINTS of the Review Paper "Classification of Methods for Determining Particle Size" published in this issue of *The Analyst* (pp. 156–187) will be available shortly from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not available through Trade Agents.

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FORMERLY THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry and of all questions relating to the analysis, nature and composition of natural and manufactured materials by promoting lectures, demonstrations, discussions and conferences and by publishing journals, reports and books.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society. If the Council in their discretion think fit, such sponsorship may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £3 3s. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No Entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The Entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st each year.

Scientific Meetings of the Society are usually held in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the official organ of the Society, which has a world-wide distribution, is issued monthly to all Ordinary and Junior Members, and contains original papers, information about analytical methods, Government reports, reviews of books and reports of the proceedings of the Society. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas. The Microchemistry, Physical Methods and Biological Methods Groups have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.

APRIL, 1963 Vol. 88, No. 1045

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 3 p.m. on Tuesday, April 9th, 1963, at Chelsea College of Science and Technology, Manresa Road, London, S.W.3. The Chair was taken by the President, Dr. D. C. Garratt, Hon. M.P.S., F.R.I.C.

The meeting consisted of contributions from Research Workers in Universities and Colleges of Advanced Technology and the following papers were presented and discussed: "The Detection of Ions in Low Concentrations Using Ion-exchange Resins," by J. F. Harvey, B.Sc.; "The Determination of Molybdenum in Several Valence States," by I. L. Marr, B.Sc.; "The Determination of Alkaline Earth Metals by Flame Spectrophotometry," by D. Spincer, B.Sc.; "The Investigation of 8-Mercaptoquinoline as a Gravimetric Reagent for Palladium and Nickel," by J. A. W. Dalziel, Ph.D., D.I.C., A.R.C.S., F.R.I.C., and D. Kealey, Ph.D., M.Sc., D.I.C., A.R.C.S.; "The Volumetric Determination of Molybdenum and Tungsten in the Presence of Iron," by M. S. Taylor, A.R.I.C.; "The Nature of the Silver (I)—Rhodamine Reaction," by A. Townshend, B.Sc.

DEATHS

We record with regret the deaths of

Frederick Mason Brewer
Alfred Henry Moore.

NORTH OF ENGLAND SECTION

A Joint Meeting of the North of England Section with the North Lancashire Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Friday, February 8th, 1963, at the Harris Technical College, Preston. The Chair was taken by the Chairman of the North Lancashire Section, Mr. T. E. V. Horsley, B.Sc., F.R.I.C.

A lecture on "Gas Chromatography" was given by A. T. James, B.Sc., Ph.D.

A Joint Meeting of the North of England Section with the Leeds University Chemical Society was held at 6.30 p.m. on Friday, February 15th, 1963, in the Lecture Theatre, The University, Leeds. The Chair was taken by the Chairman of the North of England Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

A lecture on "Dithizone and its Reactions" was given by Professor H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., L.R.A.M.

A Joint Meeting of the North of England Section with the Newcastle and North East Coast Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Wednesday, March 6th, 1963, at the Lecture Theatre, Chemistry Department, King's College, Newcastle upon Tyne. The Chair was taken by the Chairman of the Newcastle and North East Coast Section, Dr. E. P. Hart, B.Sc., F.R.I.C.

The following paper was presented and discussed: "The Chemistry of Crime Detection," by H. J. Walls, B.Sc., Ph.D.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Glasgow and West of Scotland Section of the Royal Institute of Chemistry was held at 7.15 p.m. on Friday, February 8th, 1963, in Room 24, Royal College of Science and Technology, George Street, Glasgow, C.1. The Chair was taken by the Chairman of the Glasgow and West of Scotland Section, Professor P. D. Ritchie, B.Sc., Ph.D., M.I.Chem.E., F.R.S.E.

The subject of the meeting was "Gas Liquid Chromatography" and the following papers were presented and discussed: "Recent Applications to the Study of Natural Products," by G. Eglinton, B.Sc., Ph.D.; "Applications to Reaction Kinetics," by J. H. Knox, B.Sc., Ph.D.

WESTERN SECTION

A JOINT Meeting of the Western Section and the Bristol and District Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Tuesday, February 19th, 1963, in the Technical College, Gloucester. The Chair was taken by Dr. C. G. Silcocks, B.Sc., F.R.I.C.

A lecture on "Luminescence" was given by Professor G. F. Garlick, D.Sc., F.Inst.P.

MIDLANDS SECTION AND BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Midlands Section with the Biological Methods Group and the Association of Clinical Biochemists (Midland Region) was held at 6.30 p.m. on Thursday, February 14th, 1963, in the Haworth Block, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Association of Clinical Biochemists (Midland Region), Dr. B, E, Northam, B,Sc., A.R.I.C.

The subject of the meeting was "The Microbiological Assay of Vitamin B_{12} and Folic Acid" and the following papers were presented and discussed: "The Assay of Vitamin B_{12} in Body Fluids," by Miss M. E. Gregory, M.Sc.Tech., Ph.D.; "The Assay of Folate Compounds in Body Fluids and Tissues," by A. H. Waters, M.B., B.S.; "The Clinical Interpretation of the Results of the B_{12} and Folic Acid Assays," by D. L. Mollin, B.Sc., M.R.C.S., L.R.C.P.

MICROCHEMISTRY GROUP

THE Nineteenth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, February 22nd, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year: *Chairman*—Mr D. W. Wilson. *Vice-Chairman*—Mr. R. Goulden. *Honorary Secretary*—Mrs. D. E. Butterworth, National Chemical Laboratory, Teddington, Middlesex. *Honorary Treasurer*—Mr. G. Ingram. *Members of Committee*—Dr. Cora W. Ayers, Dr. Bella B. Bauminger, Dr. R. A. Chalmers, Mr. C. B. Dennis, Mr. T. R. F. W. Fennell and Mr. C. Whalley. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Honorary Auditors.

The Annual General Meeting was followed at 7 p.m. by an Ordinary Meeting of the Group, at which the retiring Chairman, Mr. C. Whalley, B.Sc., F.R.I.C., gave an Address on "Microchemistry and the Space Age." The Chair at this meeting was taken by the new Chairman of the Group, Mr. D. W. Wilson, M.Sc., F.R.I.C.

PHYSICAL METHODS GROUP

THE eighty-seventh Ordinary Meeting of the Group was held at 6 p.m. on Tuesday, February 26th, 1963, in the Cuthbert Wallace Lecture Room, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2. The Chair was taken by the Chairman of the Group, Dr. W. Cule Davies, F.R.I.C.

The subject of the meeting was "Fractionation of High Molecular Weight Compounds" and the following papers were presented and discussed: "Gel Filtration as a Method of Polymer Fractionation," by M. F. Vaughan, B.Sc.; "Heterogeneity Studies on a Protein Hormone," by W. M. Hunter, B.Sc. (see summaries below).

The meeting was preceded at 2.30 p.m. by a visit to the Research Departments of the Royal College of Surgeons of England, Lincoln's Inn Fields and the Imperial Cancer Research Fund.

GEL FILTRATION AS A METHOD OF POLYMER FRACTIONATION

MR. M. F. VAUGHAN said that gel fractionation was a type of molecular sieving that depended on the depth of penetration of different molecules into gel-like materials. Thus, when a solution of molecules of different sizes was eluted through a column of a gel, the smallest molecules penetrated further into the gel particles, differential retardation occurred and the molecules left the column in order of decreasing molecular weight.

Some of the earliest experiments on this phenomenon had been carried out by Lathe and Ruthven (*Biochem. J.*, 1956, **62**, 665), who used columns of starch swollen with water. They were able to show that the degree of penetration of molecules into the starch granules decreased with increasing molecular size and they had indicated how this effect might be used as a separation technique. Porath and Flodin (*Nature*, 1959, **183**, 1657) had developed this idea and had shown how columns of swollen crosslinked dextrans could be used to separate water-soluble molecules of different sizes. Further improvements had been made, and it was now claimed that, by using dextrans of different degrees of crosslinking, separations could be achieved over the molecular-weight range 4000 to 2,000,000.

Crosslinked polymer structures analogous to crosslinked dextrans were fairly easily prepared and such materials as (*i*) vulcanised rubber and (*ii*) copolymers of styrene and divinyl benzene had been used in adapting this technique to non-aqueous systems. Brewer (*Nature*, 1960, **188**, 984) had used pieces of vulcanised natural rubber latex for separating polymers from mineral oil, and crosslinked polystyrene beads had been used by Cortis-Jones (*Nature*, 1961, **191**, 272) as a separation method for low molecular-weight materials and by ourselves (*Nature*, 1960, **188**, 984) at Teddington as a method for fractionating polystyrene. However, certain experimental difficulties had been encountered because, in order to fractionate the largest molecules, very lightly crosslinked beads were required. These were difficult to prepare and when swollen were so soft that they tended to pack down giving very slow elution rates. An investigation had therefore been made into the possibility of using other materials (*Nature*, 1962, **195**, 801).

The name gel filtration appeared to imply that some swollen jelly-like material was required. On the other hand, if this phenomenon really was a type of molecular sieving, then it should have been possible to use more rigid microporous materials. Some fractionation had been obtained with polyethylene gels, cellulose gels, Linde type molecular sieves, porous glass, Johns Manville firebrick, etc., but best results had been obtained with certain highly expanded silica gels.

With the silica gel Santocel A (Monsanto Chemical Co. Ltd.) the fractionation efficiencies with polystyrene had been similar to those obtained by conventional fractionation techniques. Compared with these other methods, a gel filtration method had the advantages of simplicity of operation, speed and economy of solvents. In dealing with industrial polymers, therefore, a fractionation method of this type should have been very useful as a comparatively rapid way of evaluating molecular-weight distribution curves and possibly for the large-scale processing of polymers and copolymers.

HETEROGENEITY STUDIES ON A PROTEIN HORMONE

MR. W. M. HUNTER said that most of the published evidence indicated that human growth hormone (HGH) prepared by the Raben procedure (*Science*, 1957, **125**, 883), from human pituitaries, was a homogeneous protein: sedimentation coefficient 2·5S, i.e.p. 4·9; M.W. 29,000. Hydrolysis yielded amino acids only. Investigation of the hormone in reactions with antibodies raised against the hormone in the rabbit largely confirmed the acceptance of the preparation as a single protein (Grumbach, M. M., and Kaplan, S. L., Ciba Coloquia on Endocrinology, 1962, **14**, 63). However, immunoelectrophoresis in agar gel indicated that the preparation consisted of a small family of proteins immunologically identical, but differing slightly in electrophoretic mobility.

The radio-immunoelectrophoretic method, used as an analytical tool, possessed great precision and good discrimination (Hunter, W. M., and Greenwood, F. C., *Biochem. J.*, 1962, **85**, 39P). This method had been used together with a bioassay method, the tibia line assay and amino acid analysis, to follow fractionation of HGH on starch grain electrophoresis and on columns of the crosslinked dextran 6200 Sephadex.

In free-boundary electrophoresis this preparation was homogeneous at pH 8·6 barbitone buffer (ionic strength 0·1). However, electrophoresis in starch grain resulted in partial separation of a slower peak and a faster more diffuse one (pH 8·4 barbitone sodium chloride ionic strength 0·11, 5 V per cm, 40 hours). Fractions comprising the trailing edge of the slow component and the leading edge of the faster component showed indistinguishable biological and immunological activities. Amino acid analyses (kindly carried out by Dr. J. E. Eastoe) were identical, except that the faster component had two fewer amide groups than had the slower one. The presence of two more free carboxyl groups in the faster component could account for the greater electrophoretic mobility. Either the hormone was present in the pituitary gland in an amido form and as a family of varying degrees of de-amido forms, or was produced by de-amidation during the initial extraction of the hormone in 85 per cent. acetic acid at 70° C.

G200 Sephadex was capable of distinguishing between proteins on the basis of molecular size over the approximate molecular-weight range of 20,000 to 500,000. Under three different conditions of pH, HGH was eluted as a single peak in the position expected for M.W. 29,000 (0·1 N acetic acid, 0·05 sodium phosphate, pH 7·5, carbonate - bicarbonate, pH 10·6, I = 0·1). However, in columns in borate buffers (pH 8·6, 0·05N sodium borate - 0·05N potassium chloride) a component amounting to some 20 per cent. of the starting material appeared unretarded by the dextran in a position expected for the 19S-macroglobulins. The main peak (65 per cent.) appears in the position expected for material of M.W. 29,000, with some trailing between the two. Identical distributions were achieved over the range 0·5 to 10 mg of HGH per ml applied to the column. Material from each of the two peaks re-chromatographed in borate appeared at its distinctive position.

In the analytical ultracentrifuge the same solution of HGH in borate - potassium chloride buffer yielded only a single peak of slow sedimenting material approximately S2·7. It appeared therefore that there was some unexplained interaction between the borate buffer potassium chloride, the dextran and a specific fraction of the Raben HGH. The two peaks, although they appeared to be identical in the ultracentrifuge, differed markedly when tested immunologically by a sensitive assay. It was not known whether the peaks differed in biological activity.

ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL

THE second meeting of the Panel was held at 2 p.m. on Wednesday, February 13th, 1963, at the Department of Medical Physics, The General Infirmary, The University, Leeds, 1. The Chair was taken by the Chairman of the Panel, Mr. W. T. Elwell, F.R.I.C.

A discussion on "The Problems in Design and Construction of Atomic Absorption Apparatus" was opened by Dr. J. B. Dawson. The laboratories and workshops of the Department of Medical Physics were also open for inspection.

Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Copper in Organic Matter

THE Analytical Methods Committee has received the following report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The members of the Sub-Committee concerned with the preparation of this report were Mr. W. C. Johnson (Chairman), Mr. L. Brealey,* Miss E. M. Chatt,* Dr. J. C. Gage, Dr. T. T. Gorsuch, Mr. E. I. Johnson, Miss E. M. Johnson, Mr. T. McLachlan,* Dr. R. F. Milton, Mr. E. J. Newman, Mr. W. G. Sharples, Mr. G. B. Thackray and Dr. G. E. Willis,† with Miss A. M. Parry as Secretary.

INTRODUCTION

The copper reagents in the dithiocarbamate class had an obvious claim for consideration, but, before finally adopting one of this group, the Sub-Committee considered also the merits of the more recently introduced derivatives of 1,10-phenanthroline. Of these derivatives, neocuproine (2,9-dimethyl-1,10-phenanthroline) was investigated on a practical basis in individual and collaborative experiments. Neocuproine can be used under conditions that render it virtually specific for copper, but these conditions impose a rather narrow pH range in a region where, for example, certain salts of the alkaline-earth metals might be precipitated if present in moderate amounts. The literature also refers to precipitation that has been attributed to the reagent itself or to some compound of the reagent, and this problem does not appear to have been elucidated or resolved.

Compared with the diethyldithiocarbamates, neocuproine has only two-thirds the sensitivity towards copper, whereas 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine) is said to be about twice as sensitive; the latter reagent has also been investigated in the form of its water-soluble disulphonic acid.[1] When more experience and information has accumulated on the use of these derivatives of 1,10-phenanthroline they may well be adaptable to methods for general application with some advantages over current practice. In the meantime, the Sub-Committee thought that the well established dithiocarbamates were adequate for present needs.

EXPERIMENTAL AND RESULTS

Of the several procedures in which the dithiocarbamates are used, that adopted by the International Union of Pure and Applied Chemistry[2] appeared the most acceptable. Many metals react with diethyldithiocarbamates, but, in the presence of ethylenediaminetetra-acetic acid (EDTA) and citrate, all interference except that from bismuth and tellurium is overcome by the masking effects of these complexing agents.[3] Sodium diethyldithiocarbamate is used in the I.U.P.A.C. method. Having in mind the superior sensitivity of zinc dibenzyldithiocarbamate, the Sub-Committee investigated the possibility of substituting this reagent in the I.U.P.A.C. method. It was found, however, that in the test for the presence of bismuth and tellurium, potassium cyanide destroys only a small proportion of the copper complex.

A proposal to substitute diethylammonium diethyldithiocarbamate for the sodium salt used in the I.U.P.A.C. method was made, as this reagent offers certain advantages; the aqueous solution of the sodium salt is unstable, but the diethylammonium salt can be dissolved in carbon tetrachloride to provide a much more stable reagent solution. Instead of adding aqueous sodium diethyldithiocarbamate solution to the test solution and extracting the copper complex with carbon tetrachloride, the copper is now more simply extracted by shaking the test solution with the solution of diethylammonium salt (0·1 per cent.) in carbon tetrachloride. This was found to be more convenient both in the analytical procedure and in the purification of the reagent solution. Before this modification was adopted it was subjected to collaborative tests.

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* Since resigned. † Since deceased.

To 5-g portions of white wheaten flour of breadmaking grade were added known amounts of copper as measured volumes of a standard solution, the samples were then decomposed with nitric and sulphuric acids and the added copper was determined by the method described in the Appendix, p. 256. The flour was found to contain originally about 2 p.p.m. of copper, and corrections for the original content are incorporated in the recovery figures shown in Table I.

TABLE I

RECOVERY OF COPPER

All results were obtained on the whole of the residue from the wet decomposition with the exception of those for Laboratory B, where the residue after wet decomposition was diluted to 100 ml and a 25-ml aliquot was taken

Laboratory	Copper added, µg	Copper recovered, µg
A	10·0	10·0
	10·0	9·8
	40·0	39·0
	40·0	39·5
B	10·0	12·0
	10·0	10·0
	40·0	42·0
	40·0	38·0
C	10·0	9·5
	10·0	10·0
	40·0	41·0
	40·0	40·5
D	10·0	10·7
	40·0	41·8
E	10·0	10·2
	10·0	11·5
	10·0	11·5
	10·0	9·5
	40·0	38·0
	40·0	40·0

TABLE II

RECOVERY OF COPPER IN PRESENCE OF BISMUTH

All results were obtained on the whole of the residue from the wet decomposition with the exception of those for Laboratory B, where the residue after wet decomposition was diluted to 100 ml and a 25-ml aliquot was taken

Laboratory	Copper added, µg	Bismuth added, µg	Copper recovered, µg
A	10·0	250	9·5
	10·0	250	9·8
B	10·0	250	12·0
	10·0	250	14·0
	10·0	250	10·0
C	10·0	250	10·5
	10·0	250	10·0
D	10·0	250	9·5
E	10·0	250	11·0
	10·0	250	11·5

Copper diethyldithiocarbamate is destroyed by cyanide, but bismuth diethyldithiocarbamate is not.[4] Tellurium diethyldithiocarbamate is partly destroyed by cyanide. If, therefore, the carbon tetrachloride layer containing the copper complex is shaken with cyanide and does not turn colourless, bismuth or tellurium, or both, are present. A further series of experiments was conducted with 5-g samples of the same flour to which known amounts of bismuth were added, and the special procedure for separating bismuth and tellurium described in the Appendix was followed. Copper diethyldithiocarbamate is stable during this treatment, but the bismuth and tellurium complexes are destroyed.[5]

Recovery figures for copper from these experiments are shown in Table II.

It was noted that, if the decomposition of the flour with nitric and sulphuric acids was completed rapidly, recovery of copper was variable. This was overcome by a final treatment of the residue with a little perchloric acid.

In Laboratory A, recovery experiments were carried out on a mixture of inorganic salts simulating the products from the wet decomposition of 5 g of full-cream milk powder. The solution prepared was—

Ferric alum	0·05 g
Calcium carbonate	2·0 g
Magnesium sulphate heptahydrate ..	0·84 g
Potassium dihydrogen orthophosphate ..	2·60 g
Hydrochloric acid, concentrated	50 ml
Water .. .. to	500 ml

The solution was extracted with diethylammonium diethyldithiocarbamate solution in carbon tetrachloride to remove any copper that may have been introduced with the chemicals.

To 25-ml aliquots known amounts of copper (see Table III) or of copper and bismuth (see Table IV) were added, and the solutions were heated with 5 ml of concentrated sulphuric acid until fumes were evolved. Each solution was cooled and diluted to 25 ml, and was then treated as described under "Determination of Copper," p. 257. Recovery figures for copper from these experiments are shown in Tables III and IV.

TABLE III

RECOVERY OF COPPER FROM SIMULATED PRODUCTS OF WET DECOMPOSITION OF MILK POWDER

Copper added, μg	10·0	10·0	10·0	40·0	40·0	40·0
Copper recovered, μg	10·3	10·0	10·4	40·3	40·6	40·4

TABLE IV

RECOVERY OF COPPER IN PRESENCE OF BISMUTH FROM SIMULATED PRODUCTS OF WET DECOMPOSITION OF MILK POWDER

Copper added, μg	Bismuth added, μg	Copper recovered, μg
10·0	20·0	9·7
10·0	20·0	10·1
10·0	20·0	9·8
40·0	20·0	39·5
40·0	20·0	39·0
40·0	20·0	40·0
10·0	40·0	10·7
10·0	40·0	9·7
10·0	40·0	9·7
40·0	40·0	39·5
40·0	40·0	39·0
40·0	40·0	39·5

PRECAUTIONS AGAINST FADING—

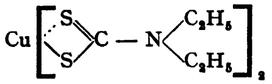
In the extraction stage it was noted that the colour of the carbon tetrachloride solution of the metal complex can fade unless the organic layer is removed from contact with the aqueous layer as soon as it has separated. A similar effect was also observed in determining copper in the presence of bismuth when the latter was removed from the carbon tetrachloride extract by shaking with N sodium hydroxide. It was found necessary to remove the carbon tetrachloride from contact with the aqueous layer as quickly as possible and to wash the organic layer immediately with water, otherwise fading occurred. Thus, after 20 minutes in contact with the sodium hydroxide solution the optical densities of two copper-containing extracts were reduced from 0·140 and 0·544 to 0·108 and 0·492, respectively. Cautionary wording against these possible sources of error has been introduced into the recommended method.

Appendix

RECOMMENDED METHOD FOR THE DETERMINATION OF COPPER

PRINCIPLE OF METHOD—

The organic matter in the sample is destroyed by a suitable method of wet oxidation. The residue is diluted with water, ethylenediaminetetra-acetate (EDTA) and citrate are added, and the pH is adjusted to 8·5 with dilute ammonia solution. The solution is then shaken with a solution of diethylammonium diethyldithiocarbamate in carbon tetrachloride. The golden-brown copper complex—



$$Cu\left[S_2C-N(C_2H_5)_2\right]_2$$

is formed and is extracted, together with those of bismuth and tellurium, if present, from the aqueous layer into the carbon tetrachloride. The organic layer is separated, and its optical density is measured at 436 mμ.

The carbon tetrachloride extract containing the copper complex is tested for bismuth and tellurium by shaking with aqueous potassium cyanide solution. If bismuth or tellurium is shown to be present, another portion of the sample solution is extracted in the usual way, but the solution of the diethyldithiocarbamates in carbon tetrachloride is washed with sodium hydroxide solution to decompose the bismuth and tellurium complexes, and the stable copper complex is determined absorptiometrically as before.

APPLICABILITY—

The method is of general applicability, but bismuth and tellurium interfere. A procedure is described that overcomes interference from these two metals.

RANGE—

The aliquot of the sample solution taken should not contain more than 50 μg of copper. The lower limit of measurement is about 2 μg, but depends on the magnitude of the reagent blank value and the type of spectrophotometric equipment available.

APPARATUS—

All glassware should be of borosilicate glass and must be thoroughly cleaned with sulphuric and nitric acids and then thoroughly washed with distilled water immediately before use.

REAGENTS—

All reagents should be of analytical-reagent grade. Reagents for the destruction of organic matter are specified in the general methods referred to under "Procedure."

Water—Use de-ionised water or water re-distilled in an all-glass apparatus for the preparation of reagents and throughout the procedure.

Ammonia solution, 6 N—Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted. Alternatively, concentrated ammonia solution "for foodstuff analysis" may be diluted with water.

Diethylammonium diethyldithiocarbamate solution—A 0·1 per cent. w/v solution in carbon tetrachloride.

EDTA - citrate solution—Dissolve 20 g of ammonium citrate and 5 g of disodium dihydrogen ethylenediaminetetra-acetate in water, and dilute to 100 ml with water. Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted.

Potassium cyanide solution—A 5 per cent. w/v solution in water.

Sodium hydroxide solution, approximately N—Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted.

Standard copper solution—Dissolve 0·393 g of copper sulphate, $CuSO_4.5H_2O$, in 2 N sulphuric acid, and dilute to 1 litre with 2 N sulphuric acid.

$$1 \text{ ml} \equiv 100\ \mu\text{g of copper.}$$

Dilute this solution with 2 N sulphuric acid shortly before use to give a working standard solution containing 2 µg of copper per ml.

Sulphuric acid, 2 N.

Thymol blue indicator solution—Warm 0·1 g of thymol blue with 4·3 ml of 0·05 N sodium hydroxide and 5 ml of 90 per cent. v/v ethanol until dissolved; dilute with 20 per cent. v/v ethanol to 250 ml.

PROCEDURE

REAGENT BLANK VALUE—

Carry out a blank test, by the entire procedure, on the precise amounts of reagents used in the test, omitting only the sample.

DESTRUCTION OF ORGANIC MATTER—

General methods for the destruction of organic matter appear in a separate report[6] and, of these, Methods of Wet Decomposition (I)A, (I)B, (I)C and (I)D, with continuation (I)(*b*)1, are suitable for use in the determination of copper, the particular method being chosen to suit the type of sample to be analysed. Dry decomposition may be preferred in some circumstances for the destruction of organic matter in the sample; the recommended method can easily be adapted to such procedures, and it is not intended to preclude such methods. The possibility of losses under some conditions of dry decomposition must, however, be kept in mind; Gorsuch[7] has demonstrated and discussed the extent of these losses.

Cool the residue from the wet decomposition, and dilute it with water. If any insoluble matter is present, filter the solution through an acid-washed filter-paper, and wash the filter-paper with water.

Dilute the solution with water to any convenient volume.

DETERMINATION OF COPPER—

Transfer 10 ml of EDTA - citrate solution and a 25-ml aliquot of the solution prepared from the decomposition residue to a separating funnel. Add 0·25 ml (5 drops) of thymol blue indicator solution and 6 N ammonia solution until the solution is green or bluish green; cool the solution before the final adjustment.

Shake the solution vigorously for 2 minutes with 15 ml of diethylammonium diethyldithiocarbamate solution, and allow the layers to separate. Place a piece of cotton-wool in the stem of the separating funnel, and, without delay, run the carbon tetrachloride layer into a 1-cm spectrophotometer cell. Take care to avoid undue exposure of the extract to light, otherwise fading may occur. Delay in separating the two layers after the extraction may also lead to fading.

Measure the optical density immediately against the reagent blank solution at 436 mµ. Convert the optical density obtained to micrograms of copper by reference to a calibration graph prepared as described under "Calibration" below.

Qualitative test for bismuth and tellurium—Transfer the carbon tetrachloride extract from the cell to a 25-ml test-tube or to a stoppered separating funnel. Add 10 ml of potassium cyanide solution, and shake. If the carbon tetrachloride layer turns colourless, bismuth and tellurium are absent.

Determination of copper in presence of bismuth or tellurium—Repeat the determination on another 25-ml aliquot of the sample solution. Transfer the carbon tetrachloride extract to a stoppered separating funnel, add 10 ml of N sodium hydroxide, and shake. After separation has taken place, remove the aqueous layer as completely as possible, repeat the washing with another 10-ml portion of N sodium hydroxide, and finally wash the carbon tetrachloride extract with 10 ml of water. Filter the extract through a cotton-wool plug inserted in the stem of the funnel. The aqueous layer should not be allowed to remain in contact with the organic layer any longer than is necessary, otherwise fading of the colour may occur; care should also be taken to avoid undue exposure of the organic layer to light.

Measure the optical density due to the copper diethyldithiocarbamate in the organic layer.

Calibration—Transfer to a series of separating funnels 10-ml portions of EDTA - citrate solution and the amounts of working standard copper solution and 2 N sulphuric acid shown below—

Working standard copper solution (1 ml ≡ 2 µg of copper), ml	0	1	2·5	5	10	15	20	25
2 N sulphuric acid, ml	25	24	22·5	20	15	10	5	0

Apply the procedure described under "Determination of Copper" to each mixture; measure the optical density of each solution against carbon tetrachloride, and construct a graph relating optical density at 436 mµ to micrograms of copper.

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The Determination of Magnesium in Aluminium Alloys by Atomic-absorption Spectroscopy*

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Aluminium seriously interferes in the atomic-absorption determination of magnesium in aluminium alloys, and a procedure is described in which 8-hydroxyquinoline is used to overcome this interference. With this reagent, interferences observed when sulphuric acid or nitric acid is present are also eliminated.

In the examination of alloys having high silicon contents a suitable compensating solution must be prepared.

It has been shown by previous workers[1,2,3,4] that the determination of magnesium by atomic-absorption spectroscopy is subject to interference, particularly from aluminium and silicon. Magnesium is, however, an important element in aluminium alloys, and its rapid determination is therefore of interest.

Various ways of avoiding the interference or of counteracting it have been suggested; Elwell and Gidley[1] use strontium, David[2] uses strontium and sulphate ions, Leithe and Hofer[3] use calcium and sulphate ions in an oxy-acetylene flame and Andrew[4] uses nickel. The amount of the reagent added to the magnesium solution is critical in most of the procedures mentioned above, and not all of them can be easily fitted into a routine composite analytical scheme for aluminium alloys. The work described in this paper represents an attempt to overcome some of these disadvantages.

It appeared, from an examination of the literature, that little work had been done to investigate the effect of organic reagents on the interference problems in atomic absorption, although Baker[5,6] demonstrated that sucrose has a strong effect on the absorption of calcium and he also looked into the effect of ethylenediaminetetra-acetic acid. Much work has been carried out on flame emission; in particular, Debras-Guédon and Voinovitch[7] investigated the action of 8-hydroxyquinoline on the emission of calcium and aluminium in the presence of fluoride and phosphate. The work described here extends the oxine technique of Debras-Guédon into the atomic-absorption field, and the findings suggest a method for determining magnesium in aluminium alloys.

DETAILS OF APPARATUS—

The equipment used in this work was a standard Hilger and Watts Uvispek, together with the Hilger atomic-absorption attachment modified to use an Evans Electroselenium atomiser. Throughout this work the normal potentiometric measuring circuit was used, no auxiliary equipment being attached.

The atomiser air-supply came from an Edwards RB.4 compressor with a stabilising tank of approximately 20-litres capacity in the air line.

Because of variations in the normal town-gas supply pressure, propane was used as fuel gas, the pressure being controlled through a single-stage regulator.

OPERATING CONDITIONS—

Preliminary work with solutions of pure magnesium established the optimum conditions listed below—

Lamp current	24 mA
Line wavelength	2582 Å
Slit width	0·1 mm
Air pressure	12 lb per sq. inch
Propane pressure	1 lb per sq. inch

It was found that the highest sensitivity was obtained when the propane pressure was 1 lb per sq. inch and the hollow-cathode lamp was so positioned that the radiation from it passed through the base of the flame just above the tips of the blue cones. It had been observed that the sensitivity increased as the propane pressure was reduced from 6 lb per sq. inch to the finally chosen value of 1 lb per sq. inch, *i.e.*, the lowest pressure for which the regulator was calibrated.

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* Presented at the meeting of the Midlands Section of the Society on Tuesday, October 9th, 1962. This paper received the Elwell Award for 1962.

EXPERIMENTAL

Initially, a calibration curve was prepared for pure magnesium over the range 0 to 20 p.p.m. (see Fig. 1, curve A). This shows good sensitivity, 0·1 p.p.m. of magnesium being easily detectable. A second calibration curve was prepared over the same range, but

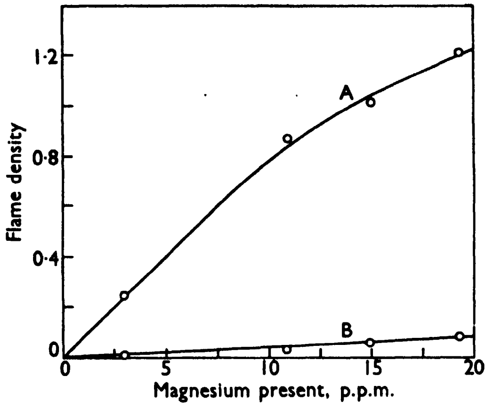

Fig. 1. Calibration curve for magnesium by atomic absorption: curve A, pure magnesium; curve B, magnesium *plus* 200 p.p.m. of aluminium.

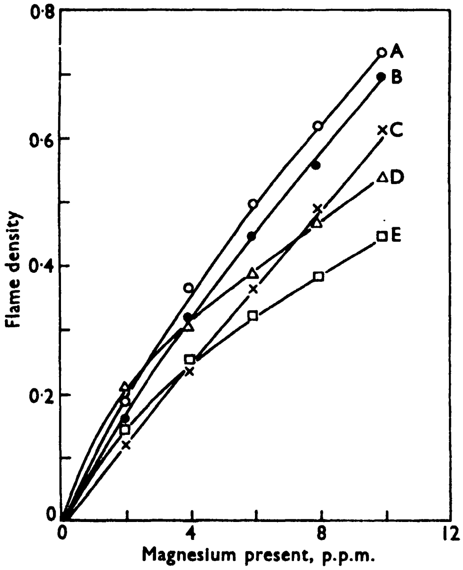

Fig. 2. Effect of various metals on the absorption of magnesium: curve A, pure magnesium; curve B, magnesium *plus* 200 p.p.m. of lead; curve C, magnesium *plus* 200 p.p.m. of copper; curve D, magnesium *plus* 200 p.p.m. of nickel; curve E, magnesium *plus* 200 p.p.m. of manganese

200 p.p.m. of aluminium were introduced into each standard magnesium solution. The resulting curve (Fig. 1, curve B) shows that 20 p.p.m. of magnesium in the presence of 200 p.p.m. of aluminium has a lower absorption than 1 p.p.m. of magnesium alone.

Fig. 2 (curves A to E) shows the effect of copper, nickel, iron, manganese and lead at similar concentrations to those quoted above.

Elwell and Gidley[1] have shown that both sulphuric and nitric acids depress the magnesium absorption, whereas hydrochloric acid has a slight enhancing effect.

To discover the effect of 8-hydroxyquinoline (oxine) on the absorption of magnesium in an aluminium - magnesium mixture, a solution was prepared so that 1 litre contained 10 g of this reagent in dilute acetic acid. This solution was then added in increasing amounts to a series of solutions that finally contained 14 p.p.m. of magnesium and 186 p.p.m. of aluminium in the presence of 10 per cent. v/v of hydrochloric acid. It was calculated that approximately 33 ml of the oxine solution would be sufficient just to complex all the metal present. The volume of solution required was not calculated more accurately because of the difficulties in preparing a reliable volumetric solution of oxine.

Additions of the solution increasing by 5-ml steps to 30 ml and then by 1-ml steps to 35 ml were made to the aluminium - magnesium solutions, which were finally diluted to 100 ml.

The resulting solutions, each containing similar amounts of magnesium and aluminium, were then sprayed, and the flame densities noted. The results of these measurements are shown in Fig. 3 (*a*) and (*b*).

To establish whether or not the increases in density shown in Fig. 3 were due to a definite effect on the mechanism of the interference or merely to the effect frequently termed "organic enhancement," a second experiment was carried out. Four series of solutions were prepared, each consisting of three solutions containing 4, 10 or 20 p.p.m. of magnesium. Nothing was added to the first series of solutions; to the second were added 10 ml of concentrated hydrochloric acid; to the third were added 10 ml of concentrated hydrochloric acid and 25 ml of 20 per cent. w/v oxine solution; to the fourth were added 10 ml of the hydrochloric acid and 50 ml of the oxine solution. The first three series of solutions were used to investigate the effects of hydrochloric acid alone and of oxine on magnesium. The fourth set of solutions containing double amounts of oxine, was used to investigate how critical was the amount of oxine added, the 50-ml addition of oxine solution corresponding to a 100-ml addition to the solution used in the preparation of the graph shown in Fig. 3. The solutions were sprayed and the flame densities measured. The resulting curves are shown in Fig. 4.

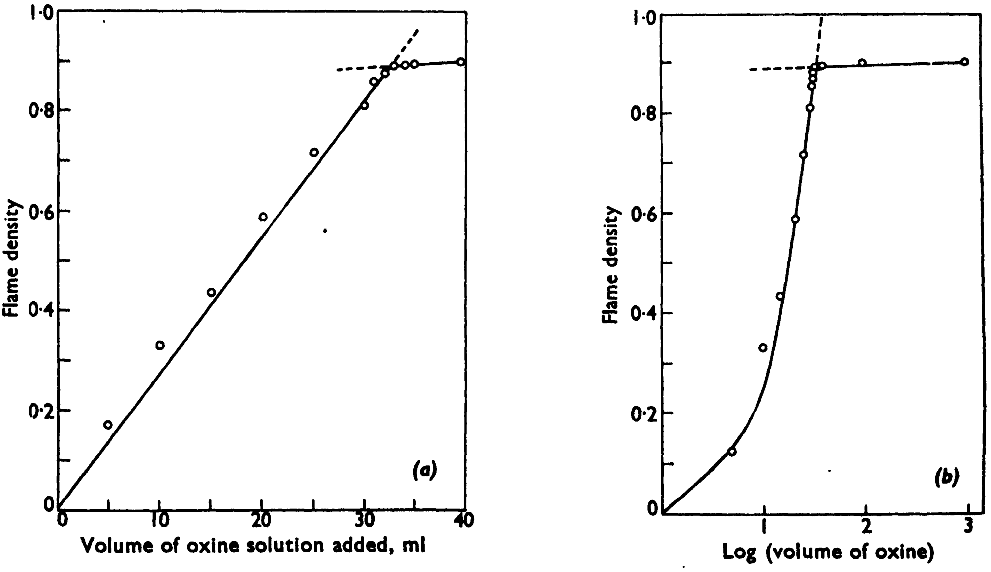

Fig. 3. Effect of oxine on magnesium in the presence of aluminium

Further investigation with solutions of pure metal indicated that all the interference shown in Fig. 2, with the exception of that by manganese, could be avoided by the use of oxine. Further, by using this technique, the effect of sulphuric and nitric acids reported by Elwell and Gidley is overcome.

Silicon was known to interfere, but it was thought that its effect could be best assessed during test analyses of alloy samples; it was considered unlikely that oxine would have any effect on the interference by silicon.

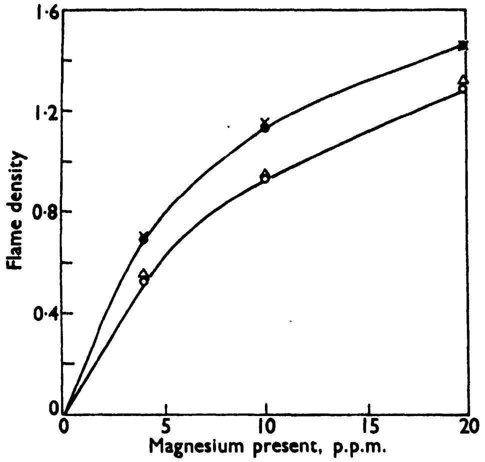

Fig. 4. Effect of oxine on pure magnesium: ○, pure magnesium; △, magnesium *plus* 10 per cent. hydrochloric acid; ●, magnesium *plus* 25 ml of oxine solution; ×, magnesium *plus* 50 ml of oxine solution

PREPARATION OF CALIBRATION CURVES—

Samples (1 g) of a series of British Chemical Standard alloys were dissolved in 40 ml of 20 per cent. w/v sodium hydroxide solution. The solution was acidified with 50 ml of 50 per cent. v/v nitric acid and boiled to dissolve precipitated hydroxides. The solution was then cooled, and diluted to 250 ml; a series of 5-ml portions was taken and additions of standard magnesium solution were made. Ten millilitres of concentrated hydrochloric acid and 25 ml of oxine solution were added, and the solution was diluted to 100 ml. The solutions were sprayed, and the magnesium absorption was measured. The resulting curves are shown in Fig 5.

It can be seen that two distinct curves are obtained. The main difference is that the standards used in the preparation of curve B had a high silicon content (11 per cent.), so that the observation made above that oxine would not counteract interference by silicon appears to be true. Curve A was prepared from alloys having silicon contents of less than 5 per cent.

ANALYSIS OF UNKNOWN SAMPLES—

Samples of aluminium alloys were analysed by the procedure described above.

For samples with magnesium contents of 4 to 9 per cent., 5-ml portions of solution were taken, and 50-ml portions were taken for samples with magnesium contents below 4 per cent. In each instance, the size of the portion used for the preparation of the calibration graph was similar to that used for the samples. It was necessary to spray standards every time analyses were carried out, because the position of the calibration curve varied slightly from day to day. This movement in the position of the curve was thought to be due to long-term variations in the atomiser and in the relative positions of the hollow-cathode lamp and the flame. The results are shown in Table I.

TABLE I

DETERMINATION OF MAGNESIUM

Sample No.	Alloy	Magnesium found by—	
		proposed procedure, %	chemical method, %
1	Duralumin-type alloy (4% of copper, <0·7% of silicon, 0·3% of iron and 0·6% of manganese)	0·60, 0·59	0·62
2		0·65, 0·66	0·66
3	B.S.S. 1490 LM 6	0·11, 0·12	0·14
4	Aluminium - magnesium - silicon alloy (<0·1% of copper, 0·7% of silicon and 0·3% of iron)	0·69, 0·70	0·64
179	Magnesium - aluminium binary alloy (B.S.S. 1490 LM 5)	4·29	4·24
182		4·52	4·50
211		4·30	4·30
218		4·20	4·20
223		3·62	3·52
314*		3·00	2·96, 2·98, 2·95, 2·96
315*		3·00	2·95, 2·91, 2·99, 3·05
316*		3·00	3·00, 3·01, 3·00, 2·86
317*		3·15	3·03, 3·05, 3·02, 3·03

* Samples analysed chemically by four different methods, including one analysis in an independent referee laboratory.

One sample (sample 321) was analysed 14 times over a period of 3 days to evaluate the reproducibility of the method. The results were 2·65, 2·60, 2·58, 2·69, 2·57, 2·59, 2·56, 2·62, 2·63, 2·58, 2·59, 2·60, 2·57 and 2·67 per cent. of magnesium, average result 2·607 per cent.; the standard deviation was 0·038 per cent. A referee laboratory obtained a result of 2·60 per cent. The result when a sulphide separation and phosphate finish were employed was 2·61 per cent. The sample was taken from a magnesium - aluminium alloy containing <0·1 per cent. of copper, <0·6 per cent. of silicon, <0·7 per cent. of iron, <0·5 per cent. of manganese and <0·1 per cent. of zinc.

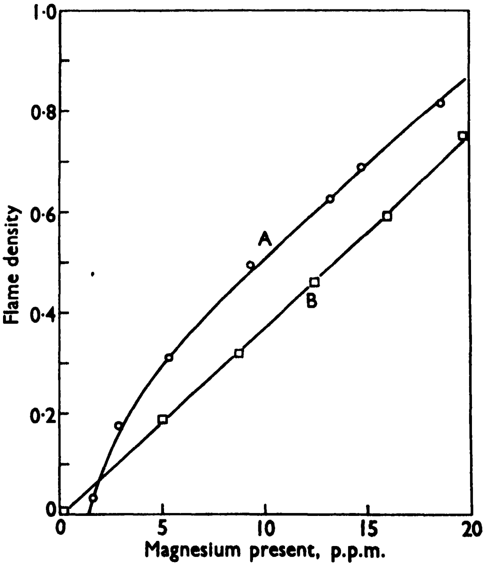

Fig. 5. Calibration curves for magnesium in aluminium alloys: curve A, low silicon alloys; curve B, high silicon alloys

METHOD

REAGENTS—

8-Hydroxyquinoline (oxine) solution—Dissolve 200 g of 8-hydroxyquinoline in 286 ml of glacial acetic acid and 200 ml of water, and dilute to 1 litre.

Sodium hydroxide solution, 20 per cent. w/v—Dissolve 200 g of sodium hydroxide in 500 ml of water, and dilute to 1 litre.

Nitric acid, 50 per cent. v/v—Dilute 500 ml of concentrated nitric acid to 1 litre.

Hydrochloric acid, concentrated.

PROCEDURE—

Dissolve a 1-g sample of aluminium alloy in 40 ml of 20 per cent. w/v sodium hydroxide solution, and, when the attack has ceased, add cautiously 50 ml of 50 per cent. v/v nitric acid. Boil the solution until all precipitated hydroxides have redissolved. Filter the solution, and dilute to 250 ml in a calibrated flask. Transfer, by pipette, a suitable portion (for magnesium contents of 4 to 9 per cent. use 5 ml, and 50 ml for magnesium contents of 0 to 4 per cent.) to a 100-ml calibrated flask, add 10 ml of concentrated hydrochloric acid and 25 ml of oxine solution, and dilute to the mark with water. Spray, and measure the flame densities three times under the conditions described under "Operating Conditions," p. 259. Calculate the average flame density.

The calibration curve should be prepared from similar aluminium alloys of known magnesium content in order to allow for the effect of silicon.

DISCUSSION OF RESULTS

The investigation shows that the addition of oxine solution to aluminium - magnesium solutions counteracts the depressive effects of aluminium on magnesium absorption. However, the curve obtained for magnesium in the presence of aluminium after the treatment with oxine is not the same as that obtained from pure magnesium solutions; there is some residual loss in density. It is clear from the results shown in Figs. 1, 2 and 3 that the effect is much more than just the frequently reported "organic enhancement," and it is closely related to the formation of co-ordinate complexes in solution.

Other work carried out in these laboratories indicates that the curve obtained from magnesium in the presence of large excesses of barium (see Fig. 6) is, when the solution is treated with oxine, similar to the aluminium - magnesium curves shown here.

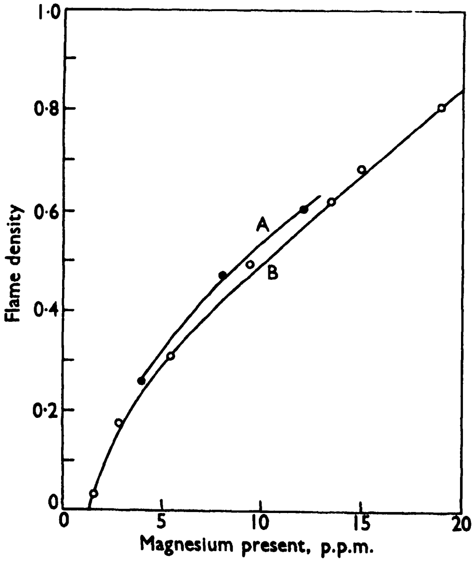

Fig. 6. Comparison of curves for magnesium in presence of: curve A, barium; curve B, aluminium

Elwell and Gidley[1] suggest a stable aluminium - magnesium compound, possibly a mixed oxide, as the cause of interference between magnesium and aluminium. They point out that the production of atoms from organo-metallic compounds is usually exothermic, but the production of metallic atoms from aqueous droplets is almost always endothermic.

Consideration of the facts reported here in the light of the above statements by Elwell and Gidley would seem to lead to the conclusion that, when the droplet enters the flame, it is dried out and disintegrates, possibly explosively, giving an organic vapour in which the magnesium and aluminium compounds are separate from one another. This vapour would then burn, and the magnesium atoms released would not be in close contact with aluminium compounds, so that no opportunity would exist for the formation of a stable mixed-oxide phase.

If this explanation is valid, it would seem reasonable to expect some evidence of the transient existence of free aluminium atoms in the flame. Debras-Guédon and Voinovitch have reported that it is possible to detect significant atomic aluminium emission from a flame into which aluminium oxinate solutions have been sprayed. This would have a definite bearing on the mechanism suggested above, and it was thought that the flame-emission experiment could profitably be repeated. It was found that the emission of the atomic line 3961 Å of aluminium was easily detectable from an oxy-hydrogen flame into which was sprayed a solution containing 200 p.p.m. of aluminium and 25 ml of oxine solution per 100 ml. This would seem to establish that simple atomic species of aluminium exist in the flame under these conditions, although their life is probably extremely short.

CONCLUSION

It is possible to counteract the interference of aluminium on magnesium atomic absorption by the use of oxine solution. The technique does not compensate for the interference by silicon, but in alloys with high silicon contents this difficulty can be overcome by using calibration standards with similar silicon contents. Alternatively, silicon can be removed by suitable chemical pre-treatment.

The method can be easily fitted into a composite scheme for determining manganese, copper, iron, nickel, titanium, zinc and magnesium in aluminium alloys on one sample weighing.

I thank the Directors of Foseco International Limited for permission to publish this paper, and Messrs. R. F. Boddey and F. E. G. Ravault for helpful discussions during the investigation.

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The Determination of Constituents of Rocks and Minerals by Gas Chromatography

Part II*. The Determination of Some Gaseous Constituents

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When rock and mineral samples are ignited in an inert atmosphere, several gaseous components are evolved. Chromatographic columns containing the appropriate stationary phase and a thermal conductivity cell have been used for separating and detecting these components. Gases that have been determined in this way include hydrogen, helium, oxygen, nitrogen and argon. The presence of carbon monoxide, carbon dioxide and hydrocarbon gases in the mixture of gases evolved on pyrolysis has also been recorded.

THAT rocks and minerals evolve large amounts of gases and vapours when heated is well known; the total volume may be many times that of the solid. Hillebrand,[1] for example, has reported that the volatile products usually comprise hydrogen and carbon dioxide with carbon monoxide, methane, nitrogen, hydrogen sulphide and other gases in smaller amounts. In recent years considerable interest has been shown in the occurrence of gaseous components of rocks and minerals. Stevenson,[2] for example, has described a method for determining nitrogen in rocks and silicate minerals, Iwasaki, Katsura and Yoshida[3] and Cuttitta[4] have described methods for determining oxygen in certain minerals and Shorokhov[5] has described a method for determining hydrogen in sedimentary rocks.

None of these methods is of general application to the determination of gaseous constituents, and none of them is easy to undertake. A somewhat simpler procedure has now been devised, based on gas-chromatographic separation and detection after evolution of the gaseous components in a small reactor attached to the chromatographic apparatus.

A similar technique has been described[6] for the gas-chromatographic determination of carbon dioxide in rocks and minerals. There the problem involved in the design of the reactor was essentially that of dealing with the high pressure developed by the liberated carbon dioxide. In the investigation described here, the problem was to design a small reactor to operate at a temperature high enough to effect complete decomposition of the sample materials in a short time.

DESCRIPTION OF APPARATUS

Initial experiments with gas burners and electric tube furnaces showed that the most satisfactory way of heating a sample was to insert it, in a platinum boat and mixed with a suitable flux, into a silica tube heated by a Meker-type gas burner. This ensured rapid and complete evolution of the gaseous constituents when samples were ground and fused as described below.

Complete expulsion of gaseous constituents is probably not achieved by direct ignition except at temperatures in excess of those permissible in an apparatus fabricated from silica. For this reason it is preferable to decompose the rock and mineral samples by fusion. Several fluxes were tried for this, but the most suitable was found to be the mixture of sodium tungstate and borax glass previously used by Jeffery and Wilson[7] for the decomposition of silicate rocks. When 100-mg portions of rock and minerals were used, decomposition was complete after fusion for a few minutes. This size of sample is sufficient for determining hydrogen and helium, although larger portions are usually required for determining the other constituents.

The reactor is shown in Fig. 1. It consists of a small silica tube, approximately 6 inches long, to which a 9/18 silica ball-joint has been fused. The spring clip supplied did not keep this joint gas-tight, and for this reason two small metal plates, with holes of the appropriate size, were bolted together across the ball-joint. With these in position and the outer part of the surface of the ground ball lightly smeared with Apiezon T grease, a gas-tight joint was obtained. The apparatus, as shown, could be improved by using silica ball-joints at both ends of the silica tube used for the ignition. The instructions given above for making the joint gas-tight would also apply to the additional joint. The three-way taps shown in Fig. 1 and the by-pass sample injector[8] were all spring loaded.

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* For details of Part I of this series, see reference list, p. 271.

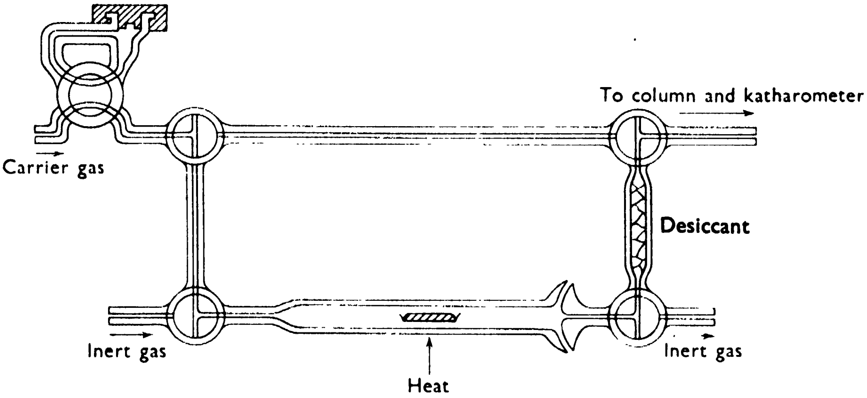

Fig. 1. Apparatus for fusion or ignition of rock and mineral samples

The desiccant is incorporated only to prevent the rapid deterioration of the molecular-sieve column, and is a coarse grade of self-indicating silica gel. Other desiccants could be employed, or the desiccant omitted altogether. If the desiccant were omitted the column material would require regeneration at intervals of about 10 days.

The remainder of the apparatus, not shown in the figure, consists of a thermal conductivity cell with associated Wheatstone-bridge network, potentiometric recorder, gas-flow control and measuring devices. These were all of the conventional type. The chromatographic column used was a 6-foot length of brass tubing (outside diameter $\frac{3}{16}$ inch) packed with molecular sieve No. 5A, ground to —44- to +72-mesh size and activated in the usual way. This column was used to separate hydrogen, helium, oxygen, nitrogen, argon and carbon monoxide. For the separation of carbon dioxide a 2-foot long column of activated charcoal was used. For separating and identifying gaseous hydrocarbons a commercial gas chromatograph was used in conjunction with a modified alumina column as previously described.[9]

The choice of carrier gas, supplied from a cylinder fitted with a two-stage regulator, depends on the constituent being determined. An inert-gas supply was also provided when hydrogen was used as carrier gas.

METHOD

GENERAL PROCEDURE—

For the decomposition of a 50-mg portion of ground rock material, weigh approximately 0·15 g of the mixed sodium tetraborate - sodium tungstate flux into a small platinum boat, and fuse thoroughly to ensure complete expulsion of all absorbed gases. Cool rapidly in a desiccator. Accurately weigh approximately 50 mg of the finely ground rock material on to the melt, and transfer the boat and contents to the silica tube. Complete the assembly of the reactor as shown in Fig. 1, and replace the gas in the apparatus by carrier gas. If the ignition is to be conducted in an inert atmosphere, replace the carrier gas in the silica tube by the appropriate inert gas.

With the carrier gas passing directly to the katharometer, heat the tube for sufficient time to ensure fusion of the sample and complete expulsion of the gaseous constituents. Allow the silica tube to cool for about 10 minutes, and then divert the flow of carrier gas to the katharometer via the ignition tube. Once the gas has been swept on to the column, restore the direct flow of carrier gas to the katharometer.

Calibrate the katharometer response by introducing portions of pure gas into the carrier gas stream by means of the by-pass sample injector, as previously described.[8]

DETERMINATION OF HELIUM—

For this determination hydrogen is used as carrier gas, and the ignition is undertaken in an atmosphere of nitrogen. No difficulty was experienced in separating helium from the excess of nitrogen present. To increase the accuracy with which the helium response could be measured, a recorder with a high chart-speed (60 inches per hour) was used. A typical chromatogram is shown in Fig. 2, and some results for a series of uranium-bearing materials are shown in Table I.

TABLE I

HELIUM CONTENT OF SOME URANIUM-BEARING ROCKS

Sample No.	Rock material	Helium found (by weight), %
M182	Pitchblende, Uluguru, Tanganyika	0·05
M229	Autunite, Katanga	0·009
M216	Torbernite, unknown locality	0·010
M230	Davidite, Mozambique	0·062
M151	Monazite, Travancore	0·016

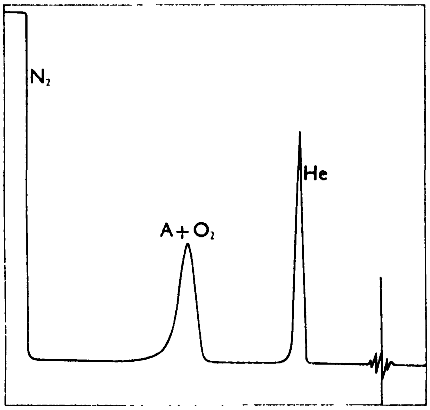

Fig. 2. Chromatogram of gases obtained from pitchblende (sample M182) with hydrogen as carrier gas and the sample fused in an atmosphere of nitrogen

DETERMINATION OF ARGON—

At normal temperatures it is not possible to separate argon from oxygen on a molecular-sieve column, and for this reason oxygen was used as carrier gas in this determination. This also reduced the amount of hydrogen evolved, and hence improved the separation of argon from hydrogen.

The apparatus shown in Fig. 1 was designed for the decomposition by fusion of samples weighing not more than 100 mg. This is insufficient material on which to determine argon. Approximate determinations have, however, been made by igniting samples of approximately 1 g in this apparatus. A typical chromatogram is shown in Fig. 3. When oxygen is used as carrier gas, the response of hydrogen and methane is in the opposite direction to that of argon and nitrogen. The small difference in thermal conductivity between oxygen and nitrogen is the reason for the insensitivity to nitrogen. The ratio of the peak areas of argon to nitrogen is, in the chromatogram shown, greatly in excess of the corresponding ratio for these gases in atmospheric air. The argon response is that of a volume of approximately 0·016 ml, representing 0·003 per cent. by weight of argon in the original material.

DETERMINATION OF HYDROGEN—

For this determination argon is used as carrier gas. The fusion of the sample material is also undertaken in an atmosphere of argon. The great difference in thermal conductivities of hydrogen and argon leads to a large chromatographic response from hydrogen. To measure

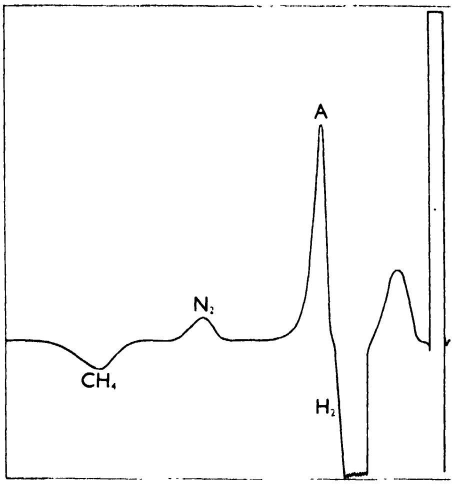

Fig. 3. Chromatogram of gases obtained from a silicate rock (sample R138; granite) with oxygen as carrier gas

this response, the device usually referred to as "backing off"[10] is used. Mixtures of hydrogen and helium would not be resolved on the 6-foot column used, but separate experiments with hydrogen as the carrier gas have shown the absence of measurable amounts of helium in all the silicate materials so far examined. Some results for a few silicate rocks are shown in Table II.

TABLE II

HYDROGEN CONTENT OF SOME ROCK SAMPLES

Sample No.	Rock material	Hydrogen content— found, by volume, %	Hydrogen content— found, by weight, %	Hydrogen content— calculated by weight,* %
R117	Granite, Shetland	55·0 65·0	0·0019 0·0022	0·005
R138	Granite, Cornwall	720 700	0·025 0·024	0·030
G-1	Granite, Westerly, R.I., U.S.A.	122 125 127	0·0042 0·0043 0·0045	0·014
W-1	Diabase, Centerville, Va., U.S.A. (Dolerite)	260 210	0·0087 0·0068	0·122

* Based on the FeO content, assuming hydrogen to be produced by the reaction—

$$H_2O + 2FeO = Fe_2O_3 + H_2.$$

There is a clear indication that the amount of hydrogen liberated is roughly reproducible for each sample of rock. In all the samples examined, the amounts of hydrogen produced were less than the amounts equivalent to the ferrous iron present, and it is therefore feasible that elemental hydrogen could be produced by reduction of water in this way. Even when sample ignition is undertaken in an atmosphere of pure oxygen, hydrogen is still recorded. The alternative explanation is that elemental hydrogen is an essential component of these materials. The experiments described have given no indication of which of these explanations is correct.

DETERMINATION OF OXYGEN AND NITROGEN—

These gases are separated on a column of molecular sieve, with argon as carrier gas. As for the determination of argon, it is usually necessary to take a large sample (0·5 to 1·0 g) if accurate peak measurements are required. Whatever the size of the sample, prolonged flushing of the reaction vessel with carrier gas is necessary to remove the entrained air. That it is possible to do this has been shown by the failure to detect oxygen in the gas evolved from certain rocks and minerals. The oxygen response appears on the trailing edge of the hydrogen response, but the separation can be improved by the introduction of a second column containing activated charcoal.

DETERMINATION OF CARBON DIOXIDE—

A well known procedure for determining carbon dioxide in rocks and minerals[11] is based on chemical absorption after direct ignition of the sample material. It is clear from the work described here that such an evolution procedure is accompanied by the liberation or production of a wide variety of carbon-containing compounds. For this reason the procedure previously described[6] (for determining carbon dioxide after digestion with phosphoric acid) is preferred to any procedure based on direct ignition of the sample material. It is clearly understood, however, that the preferred procedure will determine only that carbon dioxide readily liberated, *i.e.*, that present in carbonate minerals.

PYROLYSIS OF SILICATE ROCK MATERIAL—

As noted above, a limitation imposed by the use of apparatus made of silica is that complete evolution of gaseous constituents is probably not obtained by direct ignition at the temperatures used. When this technique is used, however, some interesting gaseous products have been detected. These include carbon monoxide, methane, ethane, ethylene and higher hydrocarbons, saturated and unsaturated. These gases are either formed during the pyrolysis or undergo reaction under these conditions, as no successive series of determinations produced any kind of agreement.

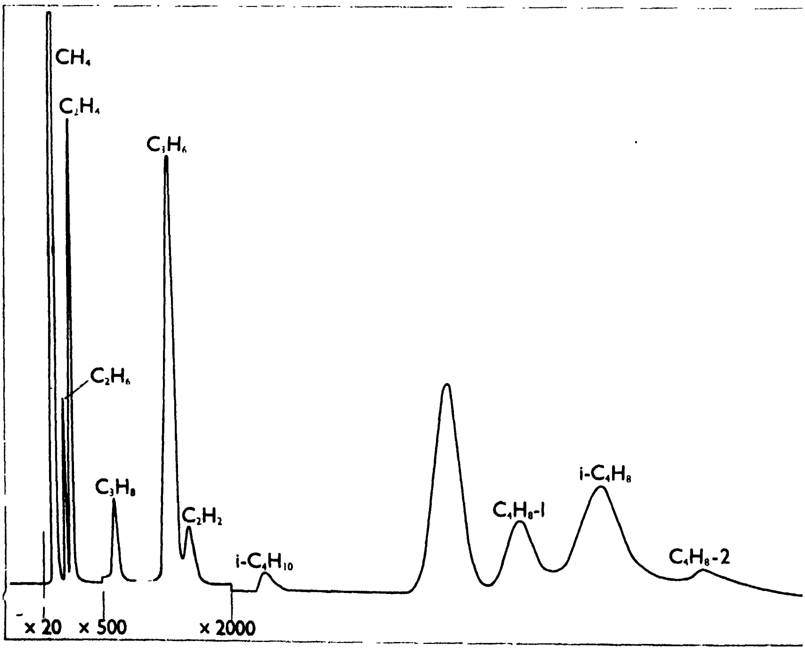

Fig. 4. Chromatogram of hydrocarbon gases obtained on pyrolysis of a silicate rock (sample R 138; granite). Separation on a column of modified alumina, hydrogen - nitrogen mixture as carrier gas and flame ionisation detector

No silicate rock has yet been examined in which carbon dioxide could not be detected, and the presence of ferrous iron in unaltered igneous silicate rocks has long been established. Carbon monoxide could therefore be formed from carbonate minerals by reduction with ferrous iron, and the hydrocarbon gases could be formed from catalytic conversion of the carbon monoxide - hydrogen mixture. The alternative explanation is that these hydrocarbons are essential components of rocks and minerals; we have not, however, been able to extract any hydrocarbons from these materials with a variety of organic solvents.

The hydrocarbons noted on pyrolysis were separated on an alumina column, suitably de-activated by adding liquid paraffin as described previously,[9] and detected by flame ionisation. A chromatogram obtained from one specimen is shown in Fig. 4.

The work described in this paper has been completed as part of a research programme of Warren Spring Laboratory, and is published by permission of the Director.

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NOTE—Reference 6 is to Part 1 of this series.

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Rapid Determination of Carbon in Steel by Infrared Gas Analysis

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A completely automatic apparatus is described for determining carbon in steel. The carbon in the sample is converted to carbon dioxide by combustion in a high-frequency induction heater. Oxygen is used to sweep the combustion products into a dry gasholder, and the gases collected are then passed through an infrared gas analyser.

With the apparatus less than 0·01 per cent. of carbon in a sample can be determined, with an error of ±0·0005 per cent., in 4 minutes.

SEVERAL methods are available for determining carbon below the 0·01 per cent. level in steel, but generally the more accurate the determination required the longer it takes to perform. It was with the aim of producing a method capable of determining less than 0·01 per cent. of carbon with an error not greater than ±0·0005 per cent. that could be completed in less than 4 minutes that the technique described in this paper was developed. It was a further aim that the operation should be simple and capable of use under routine control-laboratory conditions.

PRINCIPLE OF THE METHOD—

The carbon in the sample is converted to carbon dioxide by combustion in a Radyne 1-kW high-frequency induction heater. The combustion products are collected in a dry gasholder of known volume and then blown through an infrared gas analyser. From the concentration and volume the total carbon dioxide and hence the percentage of carbon in the sample is calculated. The gas analyser is specific for carbon dioxide and there is therefore no need to remove sulphur dioxide or water vapour from the gas stream.

Gas analysers capable of measuring sulphur dioxide are available, and it is possible to determine carbon and sulphur simultaneously by connecting two analysers in series.

A description of the principle of the gas analyser is given on p. 278.

SIMPLE FORM OF THE APPARATUS—

The high-frequency heater is available complete with the combustion chamber and sample loading mechanism. The gasholder is made from Perspex and is sold as a gas sampler. The gasholder is connected to the combustion chamber via a 3-way tap with T-shaped bore, as shown in Fig. 1. After the sample has been placed in the combustion chamber, the 3-way tap is turned so that there is a direct connection to the gas analyser. This allows oxygen to blow out any atmospheric carbon dioxide. The tap is then turned to stop the gas flow from the combustion chamber, and the high-frequency heater is switched on. The sample burns and draws all the oxygen it requires from the purified oxygen supply connected to the inlet of the combustion chamber. Progress of the combustion can be seen through the combustion chamber walls, and, when complete, the tap is turned to blow the combustion products into the gasholder. The dust filter removes any iron or flux oxides that blow out

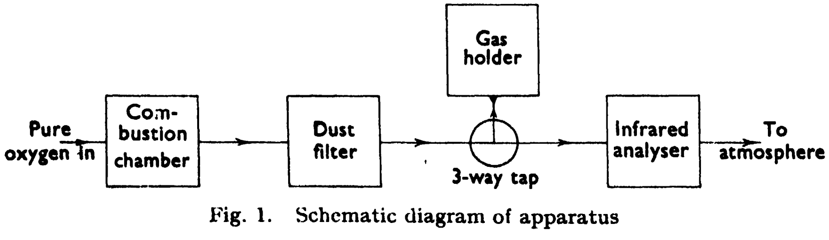


Fig. 1. Schematic diagram of apparatus

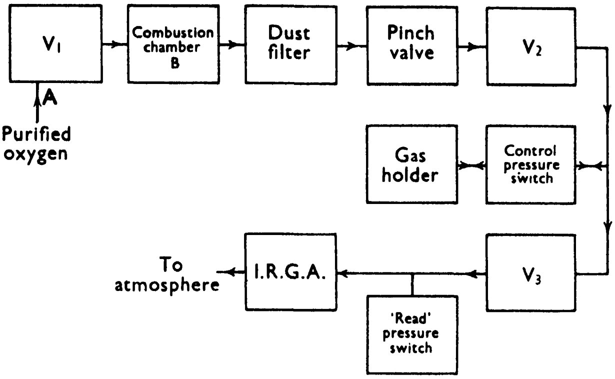


Fig. 2. Schematic diagram of gas flow

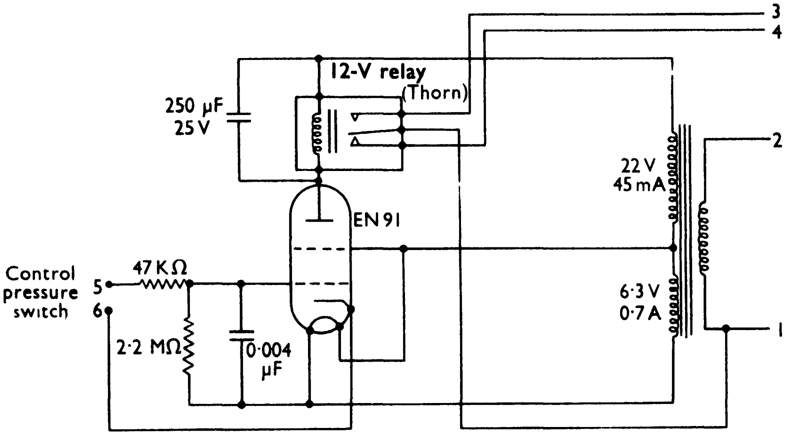


Fig. 3 (*a*). Electronic relay circuit

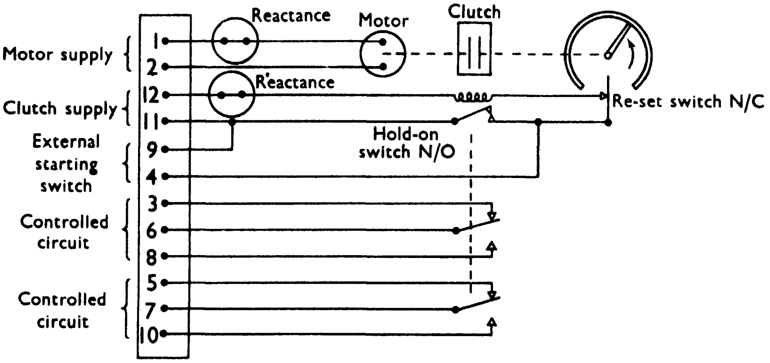
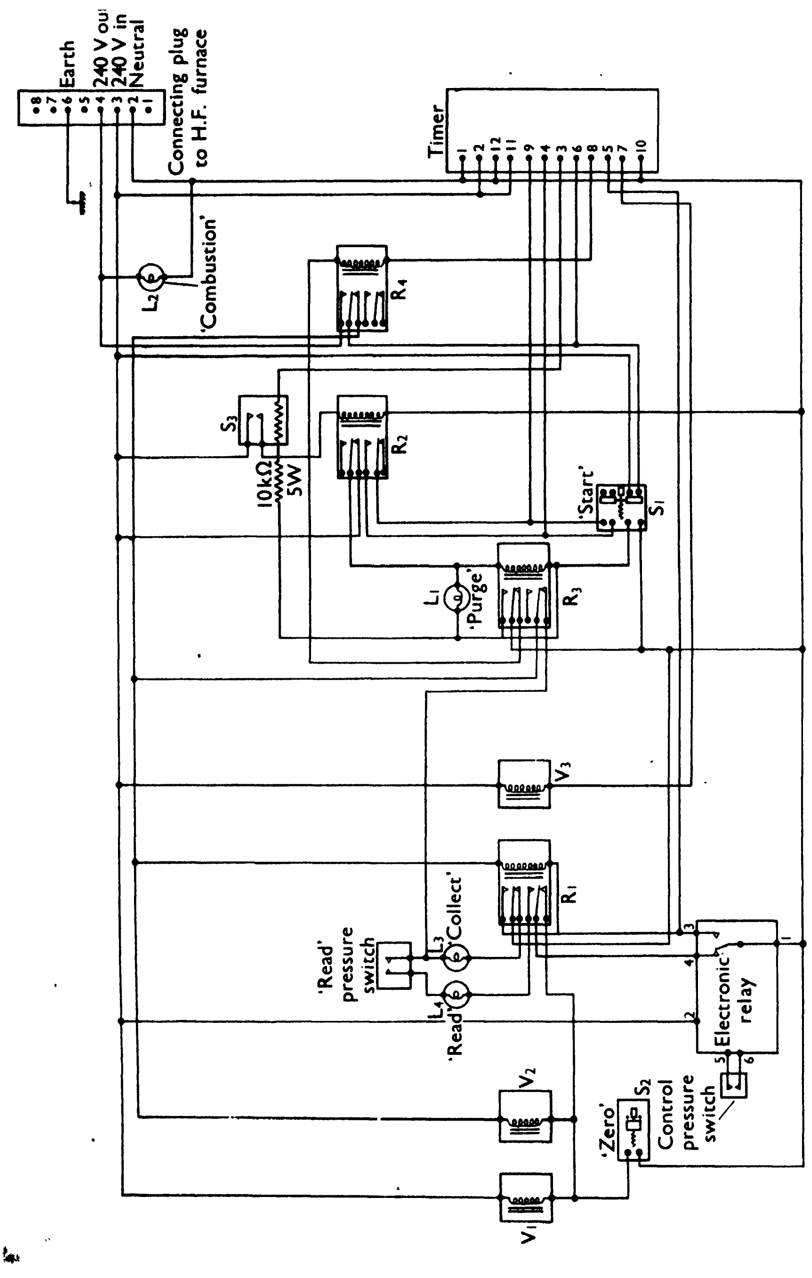


Fig. 3 (*b*). Internal connections of process timer of the combustion chamber. As the piston in the holder approaches the top, the gas flow is reduced so that there is no pressurisation. Diffusion in the holder is extremely rapid and the mixed gases can be immediately passed into the gas analyser by turning the 3-way tap. The pressure required to raise the piston to the top of the holder can be measured by inserting a gauge between the tap and the gasholder.

This technique is simple, but a more refined apparatus was required that would relieve the operator from all duties after inserting the sample. The automatic apparatus described below was therefore designed.



V₁, V₂, V₃ = 240-volt automatic controls (Black type LO/2)

R₁, R₂, R₃, R₄ = 240-volt double-pole double-throw relays (Thorn)

L₁, L₂, L₃, L₄ = 240-volt 15-watt bulbs

S₁, S₂ = Double-pole double-throw biased switches (Arcolectric T510)

S₃ = Hot-wire relay (Sunvic type 602 V)

Timer = Chamberlain & Hookham, type CA

'Read' pressure switch = Short & Mason, type 3447

Fig. 3 (*c*). Theoretical circuit of Autocarb unit

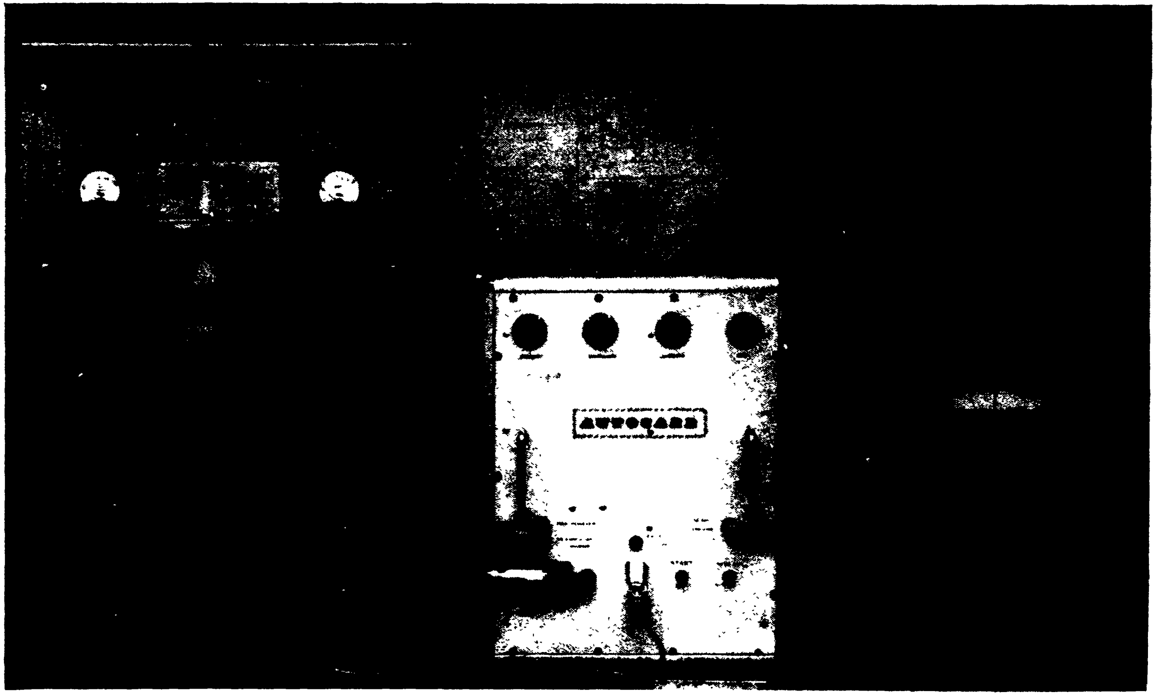


Fig. 4. General view of apparatus

Fig. 5. Rear view of control unit

AUTOMATIC APPARATUS—

Oxygen at 6 lb per sq. inch, purified by conventional means, is fed in at point A (see Fig. 2) and flows out to the combustion chamber at B after passing through valve V_1.

Valve V_1 is normally closed until the sample is placed in the combustion chamber and the start switch is depressed. V_1, V_2 and V_3 then open to allow purified oxygen to sweep out any atmospheric carbon dioxide from the combustion chamber.

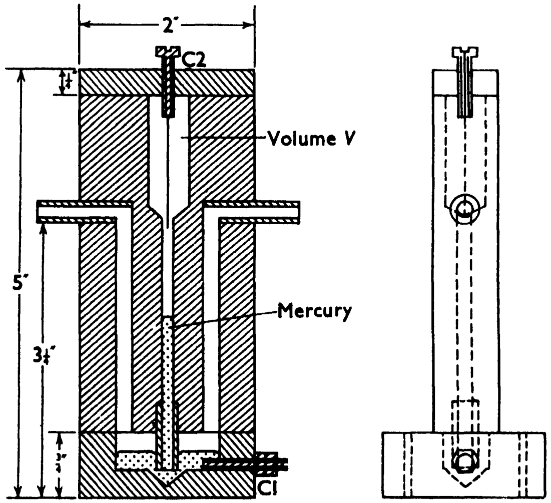
After a pre-set time, V_2 and V_3 close and the high-frequency power switches on for a second pre-set time to burn the sample. At the end of the combustion V_2 opens, and the combustion products are collected in the gasholder. When a fixed amount of gas has been collected, valves V_1 and V_2 close and V_3 opens. The gases then flow out to the gas analyser.

The process is completely automatic from when the start switch is depressed. Indicator lights show the progress of the analysis and when to take the reading from the meter, which is calibrated directly in percentage of carbon.

The electrical circuit is shown in Figs. 3 (*a*), (*b*) and (*c*). A general view of the apparatus and the rear view of the control unit are shown in Figs. 4 and 5.

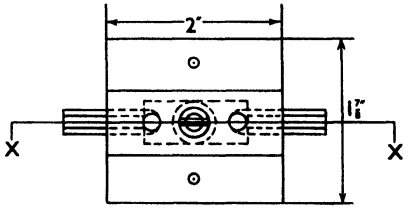
DETAILS OF PRESSURE SWITCH—

To ensure that the same number of moles of gas are collected in the holder under different conditions of temperature and pressure, a special pressure switch was constructed. The design is such that if the temperature remains constant the pressure to which the gas is collected in the holder is always the same and independent of atmospheric pressure. If the temperature alters, however, the pressure to which the gas is collected alters to compensate for the effect of the temperature on the moles of gas in the holder. Perspex block, ¾ inch thick, is used for the construction, details of which are shown in Fig. 6. The platinum contacts, C_1 and C_2, are tapped into the blocks and cemented with epoxy resin.



Side elevation section through XX

End elevation



Plan view

Fig. 6. Detail of pressure switch

As gas flows through the lower chamber of the switch it forces mercury a little way up the central column. When the gasholder piston reaches the top the pressure increases, and the mercury in the central column rises further until it makes contact with C_2 and completes the electrical circuit to C_1. The volume, v, is isolated from the atmosphere so that the pressure required for the mercury to reach C_2 is independent of atmospheric pressure. On the other hand, ambient temperature fluctuations will affect the pressure in volume v in the same sense that they will affect the pressure in the gasholder and will therefore cancel out.

The pressure to which the gas will be collected can be varied by adjustment of the initial height of the mercury in the central column.

SAMPLE WEIGHT AND RANGE OF ANALYSER—

The gas analyser can be obtained from the Infra Red Development Co., and can be calibrated to suit the requirements of the user. The instrument in the equipment described in this paper is provided with two ranges to read from 0 to 0·016 and from 0 to 0·064 per cent. of carbon when 8-g samples are taken. The range can be extended by using either smaller weights of sample or a less sensitive analyser. If it is required to use smaller samples a more sensitive analyser can be used. Sample weights of from 1 to 8 g have been used satisfactorily with this equipment.

GASHOLDER—

The volume of the gasholder is about 2½ litres, and the volume is measured accurately before use by filling it with water. This volume has been found to be more than enough to sweep out the combustion chamber. A simple check of this can be made by allowing the control unit to perform a second cycle without removing the used crucible from the combustion chamber. If any carbon dioxide were left in the chamber, the gas analyser would show a positive reading during the purge period. This does not happen.

CALIBRATION—

Two adjustments are required to calibrate the analyser.

1. Pure oxygen is blown through, and the "zero" control is adjusted to give zero deflection on the scale. A "zero" button is provided on the control unit, which when pressed will allow pure oxygen to flow through the analyser.
2. A standard mixture of carbon dioxide in an inert carrier is blown through, and the "sensitivity" control is adjusted to give the appropriate reading on the scale.

The standard gas mixture is supplied by the instrument manufacturer, but is only accurate to within ±5 per cent. of its stated value. This is a convenient way of setting up and checking the instrument provided that the cylinder of standard gas is recalibrated before use.

The most convenient method of doing this is to "inject" accurately measured volumes of pure carbon dioxide into the gas stream between the combustion chamber and the gasholder. By knowing the amount added, the volume of the gasholder and the temperature and pressure to which the gasholder is filled, it is a simple matter to calculate the percentage of carbon dioxide in the gas delivered to the analyser. The analyser can be adjusted to read this value and then used to standardise the cylinder. Once this is done the cylinder can be used for day-to-day checks of the analyser.

The accuracy of the whole apparatus will depend on the accuracy with which the small volumes of pure carbon dioxide are dispensed. Two methods have been tried and both give satisfactory results. The simpler of the two is to inject the carbon dioxide directly through the rubber tubing with a gas-tight gas-chromatography syringe. The alternative is to construct a simple gas pipette, as shown in Fig. 7, which is connected into the system as indicated. The volume between the taps is calibrated by weighing the amount of mercury

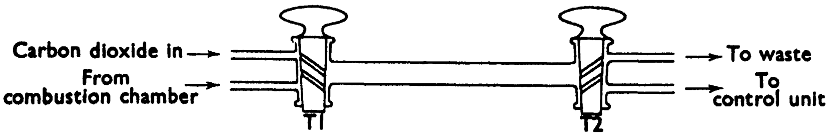


Fig. 7. Gas pipette

required to fill it and should be of such volume that about two-thirds deflection will be given on the meter. To use the pipette pure dried carbon dioxide is blown through it in the direction shown, and taps T_1 and T_2 are turned to the "off" position in that order. T_1 is then turned to connect to the combustion chamber, and the "start" button is depressed. Immediately the "collect" light comes on T_2 is turned to connect to the control unit. The measured volume of carbon dioxide is then collected and fed to the analyser.

With both methods allowance is made for atmospheric temperature and pressure.

BLANK VALUES—

It is more difficult to measure the blank value on a high-frequency combustion furnace than on a normal resistance heating tube because it is not possible to heat a crucible unless there is a sample in it. The blank value is much lower, however, because the crucible does not become as hot as an ordinary combustion boat. This does not prevent the sample itself from attaining an extremely high temperature, but the combustion is completed in a matter of seconds, so that the total heat involved is much less than in a tube furnace. The blank value is not only lower on the crucible, but also lower for the oxygen, because it does not have to pass through a hot tube.

Table I shows mean results for samples analysed by both high-frequency and resistance heating methods. No blank value for crucible or oxygen has been deducted from the high-frequency heating results. There is a favourable comparison between the results indicating that the blank value is negligible. Further, no significant difference has been detected between steel analysed when half- and full-weight samples were taken.

Blank values for tin flux can be measured by analysing standards with different weights of flux and calculating the blank value from the difference in the results obtained. By this procedure an accurate analysis of the standard is not required. The normal blank found on 1 g of tin is about 25 μg of carbon; on an 8-g sample this is equivalent to 0·0003 per cent. of carbon.

TABLE I

COMPARISON OF RESULTS OBTAINED BY DIFFERENT METHODS IN DIFFERENT LABORATORIES

	Carbon content found by— infrared method.	low pressure method. Analyst No.			gravimetric method. Analyst No.		
Sample	Analyst No. 1,* %	1,* %	2,† %	3,† %	1,* %	3,† %	4,† %
A	0·0296	0·0295	0·0306	0·0290	0·0290	0·0290	0·030
B	0·0231	0·0231	0·0244	0·0229	0·0230	0·0230	0·0240
C	0·0043	0·0041	0·0045	0·0036	0·0040	0·0039	0·0042
B.C.S. 264‡	0·0367	0·0361	—	—	0·0360	—	—

* Analyst No. 1 (the author) used high-frequency heating.

† Analysts No. 2, 3 and 4 used resistance heating. Analyst No. 2 was J. Borrowdale, R.T.B. Ltd., Scunthorpe; analyst No. 3 was C. E. A. Shanahan, R.T.B. Ltd., Aylesbury; analyst No. 4 was H. Padget, R.T.B. Ltd., Ebbw Vale.

‡ Carbon content 0·037 per cent.

TABLE II

RANGE OF RESULTS OBTAINED BY THE INFRARED METHOD

Sample	Carbon content, %
A	0·0298, 0·0298, 0·0313, 0·0298, 0·0290, 0·0294, 0·0294
B	0·0229, 0·0229, 0·0232, 0·0230, 0·0232, 0·0234, 0·0230
C	0·0045, 0·0044, 0·0042, 0·0040, 0·0042, 0·0044, 0·0044
B.C.S. 264	0·0366, 0·0366, 0·0372, 0·0374, 0·0354, 0·0371, 0·0371
D	0·0035 to 0·0043*
E	0·0370 to 0·0383†
F	0·0078, 0·0074, 0·0074
G	0·0013, 0·0013

* Range of results of 30 analyses.

† Range of results of 28 analyses.

TABLE III

CARBON DETERMINED IN VARIOUS B.C.S. SAMPLES

Sample	B.C.S. No.	Weight of sample, g	Carbon present, %	Carbon found, %
Rustless steel*	211/1	2	0·24	0·245, 0·243, 0·239
Carbon steel	237	4	0·083	0·082, 0·082, 0·083
18/8 Stainless steel	235/1	8	0·042	0·042, 0·039
Carbon steel	265	8	0·047	0·048, 0·048, 0·048
Free-cutting steel†	152/2	7	0·06	0·060, 0·061
Carbon steel	238/1	2	0·21	0·204, 0·202, 0·204
Carbon steel	240/1	1	0·45	0·444, 0·443
Pure iron granules	149/1	8	0·003	0·0025, 0·0025

* Contains 13 per cent. of chromium.
† Contains 0·26 per cent. of sulphur.

The method was primarily intended for determining carbon in silicon steels with which 1 g of tin is found to be sufficient for 8-g samples. All results quoted in Tables I, II and III were obtained with 1 g of flux, except for B.C.S. 240/1, which, even though only 1-g samples were used, was found to need 2 g of flux to obtain a satisfactory combustion.

PRINCIPLE OF THE GAS ANALYSER—

The principle of operation of the gas analyser is described in a pamphlet entitled "Gas Analysis by Infra Red Spectra," which can be obtained from the Infra Red Development Co. Limited, Welwyn Garden City, Herts. This type of analyser was originally developed by Luft before the second World War, and the general principle is as described below.

The radiation from the two heated filaments, F : F (see Fig. 8), is passed through tubes R and S and falls on to the two halves of the detector, D_1 and D_2. These halves are filled with carbon dioxide and are separated by a thin diaphragm. When equal radiation reaches the two halves of the detector the heating effect is equal and the pressure of the gas in the two halves is the same. If carbon dioxide is present in sample tube S, less heating effect takes place in D_2 and the diaphragm is deflected. To convert this deflection into an electrical signal a shutter is rotated between the sources and the tubes. This causes the beam to be pulsed so that the diaphragm oscillates. By making the diaphragm one plate of a condenser an a.c. signal can be produced, which, when amplified and rectified, can be fed to a meter. By using this sytem there is no need to monochromate the infrared radiation, because the detector can only respond to radiation in the carbon dioxide absorption band.

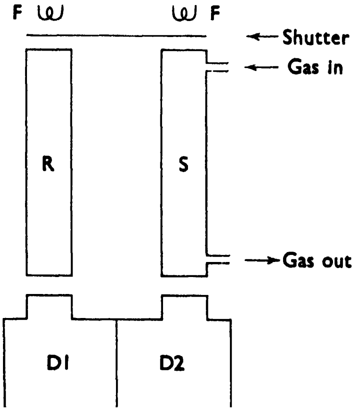


Fig. 8. Operation principle of gas analyser

RESULTS—

Table I compares results from different laboratories and by different methods. Table II shows the individual results and indicates the precision obtained. Other results on B.C.S. samples are shown in Table III.

It has been found that the method more than satisfies the objective accuracy of ±0·0005 per cent. below 0·01 per cent. of carbon. Operation of the apparatus is extremely simple, the only training required being in the preparation of sample and crucibles and the weighing of the sample.

Excluding weighing and sample preparation, the time required for drilled samples is approximately 2½ minutes before the result is obtained and a further minute before the apparatus is ready for the next sample. During this latter time, however, the next sample can be placed in the combustion chamber so that repeat analyses can be obtained in 3 minutes. Up to a minute longer may be required with chipped samples because of their slower heating in a high-frequency field.

I thank Richard Thomas & Baldwins Limited for permission to publish this paper, the Infra Red Development Co. Limited for their assistance and the other chemists in the Company who have provided the results quoted.

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The Determination of Hydrogen in Magnesium Alloys

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A method is described for determining hydrogen in magnesium alloys involving the simultaneous sublimation of magnesium from a graphite crucible and the removal of hydrogen from the furnace system. The amount of hydrogen extracted from a known weight of sample is determined by collecting the gas in a calibrated volume and measuring the pressure with a McLeod gauge before and after diffusion through a palladium tube. The results for the reproducibility tests show the coefficient of variation to be not greater than 8 per cent. for samples weighing between 0·05 and 2 g and having hydrogen contents between 6 and 140 ml per 100 g. Possible sources of error and the precautions necessary to minimise the errors are discussed.

MAGNESIUM alloys are used as a canning material for uranium fuel elements in nuclear reactors of the Calder Hall pattern. The alloys of particular interest contain 0·8 per cent. of aluminium (Magnox AL.80), 0·5 per cent. of zirconium (Magnox ZR.55) or 0·7 per cent. of manganese (Magnox MN.70). Since the presence of gases in many metals is known to affect their physical properties, a method of determining hydrogen was investigated to facilitate the assessment of its effect on these magnesium alloys. The normal level of hydrogen is 10 ml per 100 g (approximately 10 p.p.m.), rising to 150 ml per 100 g for hydrided Magnox ZR.55.

Preliminary work[1] with (*a*) a vacuum extraction procedure and (*b*) a vacuum fusion in a tin bath suffered from the long extraction times required by both methods. In order to deal with many samples, a more rapid analytical technique was needed. The vacuum sublimation method was therefore developed. In this method the sample is inductively heated at 1000° C in a graphite crucible. The magnesium sublimes on to the cooler parts of the furnace tube, and the hydrogen evolved is pumped away and measured. At this operating temperature interstitial hydrogen is released, and any hydride contained in the sample is decomposed.

METHOD

APPARATUS—

The furnace assembly (see Fig. 1) consists of a vertical silica tube, housing a graphite crucible, and a horizontal sample-loading arm; the assembly is connected to the analytical part of the apparatus via a mercury diffusion pump. The sample is moved into position by a mild-steel pusher and magnet. The graphite crucible is heated by a 1-kW 5-Mc/s high-frequency generator, and the temperature is measured with a disappearing-filament pyrometer.

The analytical system (see Fig. 2) consists of an Edwards GM2 mercury diffusion pump backing into a calibrated volume. Supplementary volumes in the form of three 1-litre bulbs may be included when large volumes of gas are being handled. Two McLeod gauges, ranges 0 to 0·1 mm and 0 to 2 mm of mercury, and a Pirani gauge are included in the system to measure the pressure before and after the hydrogen has been diffused out of the analytical system via a palladium thimble. Two cold traps are incorporated in the system, one to protect the palladium thimble, the other to prevent the mercury vapour from the GM2 diffusion pump reaching the furnace.

The whole apparatus is evacuated by an Edwards 1M2 mercury diffusion pump backed by an Edwards 1S50 rotary pump. The apparatus can measure volumes down to 0·01 μl (at N.T.P.).

PROCEDURE—

Prepare the samples (maximum of 20) by cutting with a clean grease-free hack-saw to give a sample weight in the range 0·1 to 1 g. Cut 0·5 g of magnesium as a "getter." Thoroughly abrade the surfaces with a clean grease-free file or by dry turning to a depth of several thousandths of an inch. Wash samples with carbon tetrachloride, and dry in air. Weigh, and immediately load samples and getter into the side-arm of the furnace assembly with clean forceps. Position the graphite crucible in the furnace tube, and evacuate the system. De-gas the graphite crucible at 1300° C overnight or until an acceptable blank rate has been achieved (less than 5 μl per hour of total gas at N.T.P.).

Drop the getter into the graphite crucible after its temperature has been lowered to 1000° C. De-gas the apparatus until the original blank rate is reached. Drop a sample into the graphite crucible, and de-gas as before. Measure the total hydrogen evolved by plotting the pressure of hydrogen against time and extrapolating the straight-line portion of the graph (equivalent to the blank rate) to zero time. Calculate the volume of hydrogen per 100·g of sample.

RESULTS

Samples of the different alloys (including hydrided material) in the form of strip or extruded bar were analysed. The weights of specimen in these tests varied from 0·05 to 2 g. The standard deviation and other relevant information are shown in Table I. The weight of sample taken has no significant effect on the reproducibility. All variations within a run are random; there is no evidence of a drop in hydrogen values from beginning to end of a run. Similarly, the variation in blank values determined in between analysing individual specimens was random throughout.

TABLE I

REPRODUCIBILITY OF RESULTS FOR HYDROGEN CONTENT OF MAGNESIUM ALLOYS

Material		Range of sample weights, g	Hydrogen content (at N.T.P.), ml per 100 g	No. of samples	Standard deviation, ml per 100 g	Extraction time, minutes
Magnox AL. 80	(a)	0·4 to 0·6	7·1	10	0·4	12
	(b)	0·9 to 1	6·7	11	0·4	12
	(c)	1·4 to 2·	6·8	12	0·4	20
Magnox MN. 70		0·2 to 1·	17	19	0·9	13
Pure magnesium		0·1 to 1·	15	20	0·8	12
Magnox ZR. 55	(a)	0·1 to 1	7·2	21	0·6	12
	(b)	0·1 to 0·4	8·6	16	0·6	12
		Apparatus blank rate	0·002*	11	0·001*	—
	(c)	0·1 to 0·2	140	16	6	10
	(d)	0·05 to 0·2	136	22	3	10
		Apparatus blank rate	0·004*	15	0·002*	—

* Figures for apparatus blank rate are in ml per hour.

ERRORS CAUSED BY DESORPTION OF GASES

SORBED MOISTURE OF APPARATUS—

Preliminary work on this technique indicated some gettering, since the first sample in a run always gave a much higher result. This is caused by the reaction of magnesium vapour with adsorbed moisture on the silica furnace walls.

It is essential to remove sorbed moisture from the walls of the apparatus by heating in a flame and collecting the vapour in a cold tap or by using the first specimen as a getter. A sample of Magnox AL.80 analysed by this procedure was shown to contain 38 ml of hydrogen per 100 g of alloy, but, if none of these precautions was taken, values as high as 44 ml per 100 g were obtained.

SURFACE EFFECTS ON MAGNESIUM—

Any method for determining hydrogen in magnesium is prone to errors owing to the surface contamination of the sample. Moisture reacts with magnesium at ambient temperature to produce a film of magnesium hydroxide, $Mg(OH)_2$, or hydrated oxide, $MgO.xH_2O$. When heated, these compounds release hydrogen by the reactions—

$$\begin{aligned} Mg(OH)_2 &\longrightarrow MgO + H_2O \text{ (slow)} \\ H_2O + Mg &\longrightarrow MgO + H_2 \text{ (fast).} \end{aligned}$$

Bobalek and Shrader[2] reported that water was released when an uncleaned specimen of magnesium was heated in an inert atmosphere at temperatures up to 420° C. Above 440° C only hydrogen was evolved, owing to the reaction of water with the magnesium. During the investigation described here, specimens of surface-contaminated Magnox AL.80 were heated in argon at different temperatures up to 600° C; release of gas began at approximately 400° C, and only hydrogen was detected.

Table II shows the errors inherent in the results when uncleaned samples are analysed.

TABLE II

SURFACE EFFECTS OF MAGNESIUM

Specimen	Hydrogen found (at N.T.P.), ml per 100 g	Sample preparation
A	63	No abrading of surface
B	35	Few thousandths of an inch removed from surface by filing

CONTAMINATION OF PREPARED SAMPLES BY HYDROGEN—

If abraded specimens are exposed to the atmosphere, fresh contamination of the surface occurs owing to its reaction with moisture and results in an increased hydrogen content. Six samples from the same sheet of Magnox AL.80 were abraded and left exposed in the laboratory for 3 days. Three specimens were analysed without further treatment, the remainder were re-abraded before analysis; the results (N.T.P.) were—

Hydrogen in once abraded specimens, ml per 100 g	..	17	19	20
Hydrogen in twice abraded specimens, ml per 100 g	..	13	13	13

This contamination can occur on storage, even in an atmosphere that might be presumed to be extremely dry. For example, several specimens cut from the same extruded bar of Magnox AL.80 previously machined to remove surface contamination were de-gassed by heating *in vacuo* at 400° C for 7 days. They were then stored in a desiccator containing fresh phosphorus pentoxide for various periods, and analysed without further treatment. The results are shown in Table III.

TABLE III

HYDROGEN CONTAMINATION BY DRIED AIR

Sample No.	Hydrogen found (at N.T.P.), ml per 100 g	Storage period
1	0·3	Immediate analysis
2	1	10 days
3	3·3	25 days

The equilibrium pressure of water vapour over phosphorus pentoxide is extremely low yielding an atmosphere containing less than 1 p.p.m. by volume of water. Under such condi tions no reaction with magnesium would be expected. However, in practice it is difficul to attain such dry conditions unless elaborate precautions are taken. The method of storage used in this instance, a standard desiccator containing phosphorus pentoxide, would certainly result in the moisture content of the air around the specimen being an order of magnitude greater than the equilibrium value. Moisture is continually being desorbed from the glass surface and is only slowly removed from the gas phase, the rate being determined by its diffusion in the air at normal pressure and the condition of the surface of the desiccant. This is a possible explanation for contamination of the samples during storage.

GETTERING OF HYDROGEN BY MAGNESIUM FILMS

Tests have been carried out to determine the gettering properties of magnesium for hydrogen. From other published work[3] it seemed unlikely that the sorption of hydrogen by a magnesium film at or slightly above room temperature would present a problem. The apparatus used for these experiments consisted of that shown in Figs. 1 and 2, with the exception of the furnace tube.

A modified furnace tube and standard-volume gas-addition device (see Fig. 3) was connected via a B29 cone and socket to the sample-loading arm. The furnace tube contained a graphite crucible heated by induction. The graphite crucible was de-gassed at 1300° C, and a sample of magnesium was dropped into the crucible after the temperature had been

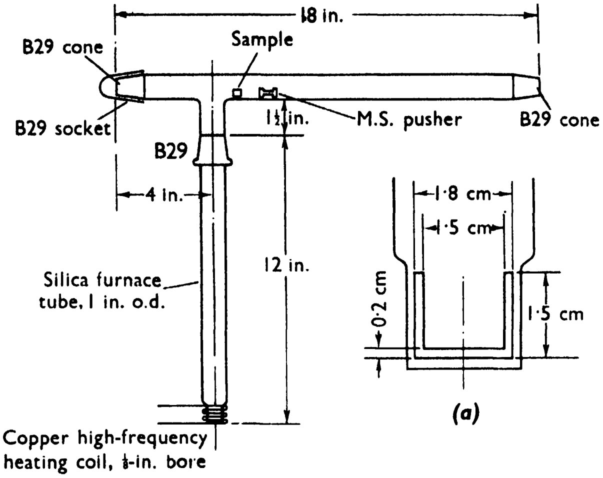

Fig. 1.′ Sample loading arm and furnace tube assembly: (*a*), enlarged view of furnace tube and graphite crucible.

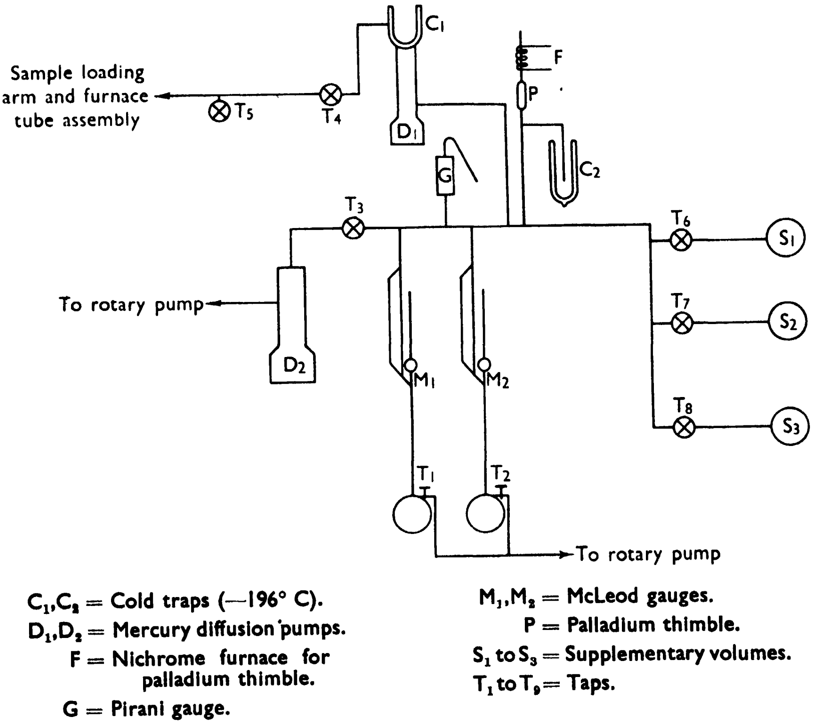
Sample loading arm and furnace tube assembly
T5
T4
C1
D1
F
P
G
C2
T3
T6
S1
T7
S2
T8
S3
To rotary pump
D2
M1
M2
T1
T2
To rotary pump
C1,C2 = Cold traps (—196° C).
D1,D2 = Mercury diffusion pumps.
F = Nichrome furnace for palladium thimble.
G = Pirani gauge.
M1,M2 = McLeod gauges.
P = Palladium thimble.
S1 to S3 = Supplementary volumes.
T1 to T8 = Taps.

Fig. 2. Schematic diagram of apparatus for extracting hydrogen in metals.

adjusted to 1000° C. Operating temperatures above 1000° C were tried, but it was observed that mechanical agitation of the sample occurred owing to rapid evolution of magnesium vapour. With Magnox ZR.55 it was feared that too rapid a sublimation might cause loss of zirconium hydride by entrainment in the vapour. This would probably lead to low results for hydrogen since (*a*) gettering of hydrogen by particles of zirconium in the cooler part of the furnace might occur and (*b*) incomplete decomposition of ejected hydride particles would be likely.

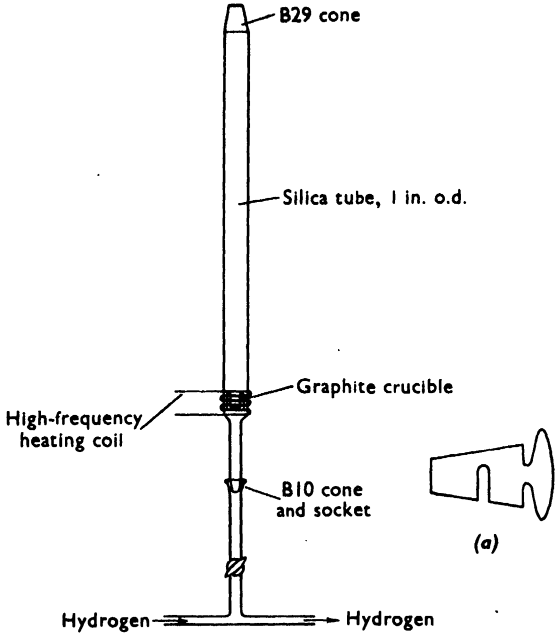

Fig. 3. Furnace tube for gettering experiments: (*a*), enlarged view of sampling stopcock.

The magnesium was sublimed from the graphite crucible on to the walls of the silica furnace tube, and the system was de-gassed to the original blank rate. A known volume (0·344 ml at N.T.P.) of hydrogen was introduced into the apparatus via the sampling stopcock at the operating temperature of 1000° C. The hydrogen flowed over the surfaces of the graphite crucible and magnesium film, and was pumped into the analytical system where it was measured. This procedure was then repeated, so as to simulate conditions of analysis. In both experiments all the hydrogen was recovered within 1 minute.

In the second test the same volume of hydrogen was introduced into the furnace after it had been isolated from the analytical system. After the hydrogen had been in contact with the magnesium surfaces for 5 minutes, the gas was pumped away, collected and measured in the normal manner; this experiment was repeated. All the hydrogen was recovered within 1 minute.

GETTERING OF HYDROGEN BY MAGNESIUM VAPOUR—

Although the experiments described above indicated that hydrogen was not removed by a pre-formed film of magnesium, the possibility of its removal by condensing magnesium vapour could not be disregarded. This was examined with the same apparatus and procedure as were used in the previous gettering tests, except that (*a*) the sample was introduced into the cold de-gassed crucible and (*b*) a known amount of hydrogen was added to the furnace system before the magnesium was sublimed. The magnesium was then volatilised in the presence of hydrogen. After a period of 5 minutes the gas was pumped away and measured. In none of the three experiments was less than 97 per cent. of the hydrogen recovered.

DE-GASSING OF ZIRCONIUM RESIDUES

Interstitial hydrogen is released by subliming magnesium from a graphite crucible. With magnesium - zirconium alloys a residue of zirconium hydride remains in the crucible. An experiment was therefore devised to determine the optimum temperature for the rapid extraction of hydrogen from zirconium. A known weight of zirconium purified by the iodide process was de-gassed at 1050° C for 5 hours, after which time the hydrogen content had been reduced to negligible proportions. A measured volume of hydrogen was then introduced into the extraction apparatus and was allowed to react with the zirconium at a temperature of 800° C. When the reaction was complete the specimen of zirconium was cooled to room temperature, and the residual hydrogen was measured. The zirconium was then heated again to 1050° C under high vacuum; recovery of the added hydrogen was 100 per cent. The results of several tests show that a temperature higher than 1050° C would be advisable for rapid extraction of hydrogen from the massive zirconium. However, when magnesium is present as in Magnox ZR.55 rapid evolution of vapour at temperatures in the region of 1200° C can cause loss of zirconium by entrainment; it was therefore decided to carry out the operation at 1000° C.

MODE OF SUBLIMATION

Two different procedures have been used for subliming magnesium. In the original experiments[1] the crucible and furnace tube were heated at 750° C (initial de-gassing at 1000 °C) by use of an external Nichrome-wire resistance element. A high de-gassing rate and severe attack of the silica furnace tube by magnesium resulted from this method of heating. The second method, high-frequency heating, permits operating temperatures of 1000° C (initial de-gassing at 1300° C) to be attained. Under such conditions, only a small proportion of the furnace tube is heated by radiation from the crucible; the blank rate is much lower and the service life of the silica tube is increased twenty-fold.

Reproducibility tests on a sample of Magnox AL.80 show the superiority of the high-frequency heating procedure (see Table IV).

TABLE IV

RESULTS WITH DIFFERENT METHODS OF HEATING

Mode of heating	Nominal hydrogen content, ml per 100 g	Standard deviation, ml per 100 g	No. of determinations
Resistance (750° C)	10	2	12
High frequency (1000° C) ..	10	0·4	11

PREPARATION OF HYDROGEN STANDARDS

A 5-g sample of de-gassed Magnox AL.80 cut from ⅝-inch diameter extruded bar was heated for 1 hour at 350° C and a further hour at 500° C in an atmosphere of hydrogen at a pressure of 3½ mm of mercury. No detectable decrease in the hydrogen pressure was observed; a repeat experiment carried out at a pressure of 7 mm of mercury at 400° C confirmed this.

A sample of Magnox AL.80 similar to that used in low-pressure hydrogenation was heated in the temperature range 350° to 550° C at a pressure of 2 atmospheres of hydrogen. Some absorption of hydrogen by the Magnox AL.80 occurred, and this was detected by a differential oil manometer. Results are shown in Table V.

TABLE V

SORPTION OF HYDROGEN BY MAGNOX AL.80

Temperature, °C	Time, hours	Pressure drop, mm of oil	Original pressure, atmospheres of hydrogen
350	½	Nil	2
400	1	1	2
450	½	5	2
550*	1	19	2

* Some volatilisation occurred.

The volume of the system containing the hydrogen was 270 ml, and the specific gravity of the oil was 0·89. Approximately 0·4 ml of hydrogen (at N.T.P.) was absorbed by the 5-g sample of Magnox AL.80 at 550° C, which is equivalent to 8 ml per 100 g. This pick-up of hydrogen was not high or rapid enough for preparing reliable standards, especially as some volatilisation of the sample occurred.

Unsuccessful attempts were made to introduce hydrogen at low pressure (3 mm of mercury) into Magnox AL.80 by activating the hydrogen with an electrodeless discharge produced by a 20-Mc/s high-frequency generator.

The tests described above were repeated with Magnox ZR.55, and the results were similarly disappointing.

No satisfactory method of preparing standards has been found.

CONCLUSION

The rapid extraction of hydrogen from magnesium alloys by the sublimation of magnesium at 1000° C is a convenient routine method. Results obtained with high-frequency heating of the crucible at 1000° C are superior to those obtained with external resistance heating at 750° C. Under the conditions described, reproducibility is good, although the surface of the samples and apparatus must be carefully prepared otherwise they can cause significant errors. The weight of sample does not affect the reproducibility of the method. Small specimens permit an increased number to be loaded into the apparatus, and therefore are preferable.

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A Dithizone Method for determining Zinc in Organic Material

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A rapid and selective one-colour dithizone method is described for determining zinc in food products or other organic material.

After wet oxidation of the sample, an aliquot is treated with cyanide in alkaline solution to mask cobalt and is then extracted with a solution of dithizone in carbon tetrachloride at pH 5·5 in the presence of thiosulphate, cyanide and citrate. All the zinc and some cadmium are dissolved as dithizonates in the carbon tetrachloride, whereas other metals that can form dithizonates soluble in carbon tetrachloride are masked by the thiosulphate or the cyanide and remain in the aqueous phase. The dithizonate solution is shaken with a solution of sodium sulphide buffered with borate in order to remove cadmium dithizonate, excess of dithizone and dithizone oxidation products. The optical density of the zinc dithizonate solution is measured at 535 mμ.

IN 1960 Margerum and Santacana[1] published an excellent critical examination of 8 different methods for determining microgram amounts of zinc. They stated that Butts, Gahler and Mellon's[2] method with bis(2-hydroxyethyl)dithiocarbamate, cyanide and citrate as masking agents was superior to the other procedures compared. The method described here, which is a development and simplification of the procedures proposed by Fischer and Leopoldi[3] and Baggot and Willcocks,[4] is more rapid for routine analyses and gives yields of zinc dithizonate as good as those obtained by Butts, Gahler and Mellon's method.

The proposed method differs from Fischer and Leopoldi's and Baggot and Willcocks's methods in, among other things, the use of one combined buffer and masking agent solution containing sodium citrate and citric acid as buffer, cyanide to mask nickel and cobalt and a large amount of sodium thiosulphate to mask other metals. The pH of the solution is 5·5, and it is the only reagent solution used that has to be purified. The amounts of masking agents have been chosen to be sufficiently large to allow considerable amounts of foreign metals to be present (see Table I), but not so large that the formation of zinc dithizonate is affected (see Table III). Only cobalt has to be masked at a pH above 5·5 and necessitates extra additions of cyanide and citric acid solutions.

Baggot and Willcocks remove excess of dithizone, dithizone oxidation products and remaining traces of cadmium dithizonate with a sulphide solution buffered with phosphate at pH 11; the phosphate buffer is purified by tedious extractions with dithizone solution. In the proposed method a sulphide solution buffered with borate (pH 11·5) is used, which gives smaller blank values than does the phosphate-buffered solution, despite the fact that it is not purified.

METHOD

The laboratory should be free from dust and, if possible, be used only for determinations of trace metals. As zinc is an extremely common metal, it is necessary to work with scrupulous care to ensure low and constant blank values and good results.

GLASSWARE—

All dishes and storage bottles must be of zinc-free borosilicate glass. Before use they should be washed with diluted hydrochloric acid (1 + 1) and metal-free water. The Kjeldahl flasks used for digestion of the organic material, if they are new or have previously been used for other analyses, should be boiled with concentrated sulphuric and nitric acids for half an hour, and then washed with metal-free water. Immediately before use all glass articles should be shaken with a mixture of 10 ml of a 0·01 per cent. solution of dithizone in carbon tetrachloride and 25 ml of 0·02 N ammonium hydroxide. This procedure must be repeated until the glass is free from adsorbed metal ions, *i.e.*, until the carbon tetrachloride solution does not show any trace of red colour. The residues of the solutions should then be removed by rinsing with carbon tetrachloride and metal-free water. It is recommended that the same glassware should be kept for all determinations of zinc and not be used for other determinations. After use the glassware should be cleaned with metal-free water, and Kjeldahl flasks and separating funnels should be stored stoppered.

REAGENTS—

All reagents should be of recognised analytical grade, and only metal-free water (see below) should be used.

Ammonium hydroxide, concentrated—Distil 1 litre of ammonium hydroxide, density 0·91 g per ml, into 500 ml of water at <10° C until the density of the distillate is 0·90 to 0·91 g per ml. (Ammonia gas from a cylinder, if available, can also be used. It should be purified by passage through a gas washing bottle containing concentrated ammonium hydroxide and then passed into cooled water until a solution of the right density is obtained.)

Ammonium hydroxide, N—Dilute 75 ml of the concentrated ammonium hydroxide to 1 litre with water.

Ammonium hydroxide, 0·02 N—Dilute 10 ml of the N ammonium hydroxide to 500 ml with water.

Buffered masking agent solution—This solution should be prepared under an efficient extraction hood. Dissolve 715 g of sodium thiosulphate pentahydrate, 60 g of trisodium citrate dihydrate and 10·0 g of potassium cyanide in 1 litre of water. Add, with stirring, a solution of 15·0 g of citric acid in 100 ml of water. Divide the solution into two portions, and shake each portion thoroughly for several minutes in a separating funnel twice with 50-ml portions of 0·04 per cent. dithizone solution and twice with 50-ml portions of 0·01 per cent. dithizone solution. Each time the layers should separate cleanly. Shake the last carbon tetrachloride layers with 15 ml of buffered sodium sulphide solution (see below). If the carbon tetrachloride layers are red or pink, repeat the extractions with dithizone and sulphide solutions until the carbon tetrachloride turns colourless (or pale green or yellow). Then wash the remaining dithizone from the two portions of masking agent solution with two or three portions of carbon tetrachloride. Combine the masking agent solutions, filter, and make up to 2 litres. The pH of the solution should be 5·5. Do not use the solution when it is more than 1 month old.

Citric acid solution—Dissolve 33 g of citric acid monohydrate in water, and make up to 500 ml.

Cotton-wool—Wash cotton-wool with dilute dithizone solution and carbon tetrachloride.

Dithizone stock solution, 0·04 *per cent. w/v*—Dissolve 0·200 g of dithizone in 500 ml of carbon tetrachloride, and filter into a dark flask. Store the solution in a refrigerator.

Dithizone extraction solution, 0·01 *per cent. w/v*—Dilute 100 ml of dithizone stock solution with 300 ml of carbon tetrachloride. Store this solution in a dark flask in a refrigerator for not more than 2 days. Allow the solution to reach room temperature before use.

Methyl red solution—Dissolve 0·04 g of methyl red (sodium salt) in 100 ml of water.

Potassium cyanide solution—Dissolve 5·0 g of potassium cyanide in water, and make up to 100 ml. The solution should not be stored for more than 1 month.

Buffered sodium sulphide solution—Pass hydrogen sulphide into 500 ml of 0·25 N sodium hydroxide until the pH is just below 8, as measured with thymol blue indicator. Do not use if it is more than 1 month old.

Dissolve 61·8 g of boric acid and 40·5 g of sodium hydroxide in water, and make up to 1 litre. The pH of the solution should be 11·5.

Before use, mix 10 ml of the sodium sulphide solution with 90 ml of the borate buffer solution.

Thymol blue solution—Grind 100 mg of thymol blue with 4·3 ml of 0·05 N sodium hydroxide in a mortar until the solid has dissolved, and make up to 100 ml with water.

Water—Pass distilled water through a column of cation-exchange resin and then do not allow it to come into contact with objects from which it can pick up metal ions, *e.g.*, certain plastic or rubber tubes. The water can be examined for metal ions by shaking 25 ml with 0·5 ml of N ammonium hydroxide and 10 ml of dithizone extraction solution; the carbon tetrachloride layer should not be red or pink.

Standard zinc solution—Dissolve 0·1000 g of zinc metal in 10 ml of diluted hydrochloric acid (1 + 1), and make up to 1 litre with water. Prepare a solution containing 2 μg of zinc per ml by diluting 20·00 ml of the stock solution to 1 litre.

PROCEDURE—

Digestion—Digest the sample, containing 5 to 100 μg of zinc, by any suitable wet-oxidation method.[5] Concentrate the acidic solution to 2 to 3 ml, and make up to 50·0 ml with water.

Separation and colorimetric determination of zinc—The analyses should be carried out under an efficient extraction hood. Transfer 10·00 ml of the sample solution to a 125-ml separating funnel, and adjust the pH to 5·5 (at room temperature) by adding ammonium hydroxide solution (concentrated and N) with methyl red as indicator. Add 1·0 ml of potassium cyanide solution, mix, and dilute with water to 25 ml. Add 25 ml of buffered masking agent solution and 1·0 ml of citric acid solution. (If the samples do not contain cobalt, the addition of potassium cyanide solution and citric acid solution can be omitted.) Ensure that the colour of the indicator corresponds to pH 5·5. Add 15·00 ml of dithizone extraction solution, and shake the funnel vigorously for 2 minutes. The funnels should be ventilated through the neck, not through the stopcock.

When the layers have separated, transfer the carbon tetrachloride layer to a 125-ml separating funnel containing 15 ml of buffered sodium sulphide solution, and shake the funnel for about 30 seconds. After the layers have been separated, filter the carbon tetrachloride layer through cotton-wool into a test-tube, and keep the tube stoppered until the optical density of the solution has been measured at 535 mμ (1-cm cell) against carbon tetrachloride. This zinc dithizonate solution should be protected from direct sunlight, and, if its optical density is not measured within 2 hours, the solution should be stored in a dark place.

Blank test—For each determination of zinc carry out at the same time a blank digestion on the same amounts of oxidising agents as are needed for digesting the sample. Take the solution from the blank digestion through the entire procedure, and measure the optical density of the final solution; subtract this value from that obtained for the sample. The optical density of the blank solution (1-cm cell) does not usually exceed 0·120.

Calibration curve—In each of six 125-ml separating funnels place 0, 2·00, 4·00, 6·00, 8·00 or 10·00 ml of standard zinc solution. Carry out the proposed procedure on these standard samples from the addition of 1·0 ml of potassium cyanide solution. Subtract the optical density for the sample without zinc from the values for the other standard samples, and plot the corrected optical densities as a function of the zinc content. A straight line passing through origin and relating 1·0 μg of zinc to an optical density of 0·096 (1-cm cell) is obtained. If the reagents are good, the optical density measured in a 1-cm cell for the sample without zinc should not exceed 0·070.

INTERFERENCES

Foreign metals—Appreciable amounts of foreign metals can be present without seriously affecting the determination of zinc (see Table I). The effects of amounts of foreign metals larger than 5 mg have not been tested.

Only small amounts of ferrous salts may be present, but this is unimportant as iron is oxidised to the tervalent state during digestion.

Effect of light—Zinc dithizonate is decomposed when its solution in carbon tetrachloride is exposed to direct sunlight. In diffuse light the solution does not change noticeably in 1 to 2 hours; no effect was observed after 24 hours in the dark (see Table II).

TABLE I

EFFECT OF FOREIGN METALS ON THE DETERMINATION OF ZINC

		Zinc found				Zinc found	
Foreign metal	Amount present, μg	No zinc added, μg	8·0 μg of zinc added, μg	Foreign metal	Amount present, μg	No zinc added, μg	8·0 μg of zinc added, μg
Fe^{3+}	5000	0·15	8·0	Cr^{3+}	5000	0·1	8·0
Fe^{2+}	250	0·0	8·0	Co^{2+}	5000	0·1	7·9
Ni^{2+}	5000	0·1	8·0	Sn^{2+}	1000	0·05	7·6
Cu^{2+}	5000	0·2	8·0	Sn^{4+}	1000	0·0	7·75
Mn^{2+}	5000	0·1	8·05	Bi^{3+}	1000	0·1	7·75
Ag^{+}	5000	0·3	8·0	Cd^{2+}	1000	0·05	7·75
Pb^{2+}	5000	0·1	7·9	Hg^{2+}	1000	0·15	7·8

TABLE II

EFFECT OF LIGHT ON OPTICAL DENSITY OF ZINC DITHIZONATE SOLUTIONS

Optical density of zinc dithizonate solution (535 mμ; 1-cm cell)				
Immediately	After 24 hours in the dark	After 1½ hours in diffuse light	After 4 hours in diffuse light	After 1½ hours in sunlight
0·836	0·837	0·837	0·784	0·383
1·030	1·030	1·026	0·970	0·528

TABLE III

EFFECT OF MASKING AGENTS ON RECOVERY OF ZINC

	Zinc found—	
Zinc added, μg	at pH 5·5 with masking agent, μg	at pH 5·5 without masking agent, μg
6·0	6·05	6·1
8·0	8·0	8·05
10·0	10·0	10·0

CONTROL OF THE METHOD

Masking agents—No loss of zinc was caused by the masking agents used (see Table III).

Stability of zinc dithizonate solution in contact with borate-buffered sulphide solution—Margerum and Santacana[1] have shown with zinc-65 that no loss of zinc is caused by shaking a dithizone - zinc dithizonate solution with a solution of sodium sulphide. The results in Table IV show that this is also true when a borate-buffered sulphide solution is used.

TABLE IV

RECOVERY OF ZINC IN PRESENCE OF BORATE-BUFFERED SULPHIDE SOLUTION

Zinc added, μg	Time of shaking, minutes	Borate-buffered sulphide solution present, ml	Zinc found, μg
8·0	½	10	7·95
8·0	½	20	8·05
8·0	½	30	8·0
8·0	½	15	8·05
8·0	1	15	8·10
8·0	2	15	8·05
8·0	3	15	8·15

Effect of pH on the formation of zinc dithizonate—Irving, Bell and Williams[6] have shown that, at pH 3·88, shaking for 30 minutes is necessary to achieve equilibrium in the reaction of aqueous solutions of zinc salts with dithizone in carbon tetrachloride. With increasing pH the rate of reaction increases and, at pH 4·9, equilibrium is reached within a few minutes.[6] Thus the rate of reaction at pH 5·5 should be satisfactory. Table V indicates that the extraction of zinc is complete by the proposed method (excess of dithizone in carbon tetrachloride; pH 5·5; time of shaking 2 minutes), as, even at pH 7·5 without masking agents (time of shaking 2 minutes) the amount of zinc extracted is not appreciably greater.

TABLE V

EFFECT OF pH ON RECOVERY OF ZINC

	Zinc found—		
Zinc added, μg	at pH 5·5 with masking agent, μg	at pH 7·0 without masking agent, μg	at pH 7·5 without masking agent, μg
8·00	8·0	8·05	8·05
10·00	10·0	10·05	10·0

VARIATION OF THE METHOD

If the sample to be analysed contains so much zinc that a portion containing 100 μg of zinc is too small to be representative, a larger portion must be taken. A smaller aliquot can then be used or the sensitivity of the method can be reduced by increasing the volume of dithizone solution used for extraction. In the latter instance only part of the dithizone - dithizonate solution is shaken with an equal volume of buffered sulphide solution.

I thank Miss Linnéa Eriksson and Mrs. Judith Antonsson for valuable technical assistance.

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Hydroxocobalamin: Its Examination and Determination in Parenteral Injection Solutions by Paper Chromatography

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The separation of hydroxocobalamin (aquocobalamin salt) from its analogues and decomposition products by conversion to the corresponding cyano-compounds, with subsequent paper chromatography, is described; coloured impurities are eluted and determined spectrophotometrically. The method can be applied to the solid material or solutions for parenteral injection after extraction to eliminate electrolytes and other interfering substances. Decomposition during storage produces additional coloured impurities, and paper chromatography in conjunction with spectrophotometry can be used as an alternative to microbiological assay during stability trials on parenteral injection solutions.

IN an earlier paper[1] it was shown that any "red acids" and neutral cobalamins that may occur as impurities in commercial hydroxocobalamin could be separated by chromatography on basic and neutral celluloses and then determined spectroscopically. Other possible impurities that cannot be separated in this way include analogues containing nucleotide bases other than 5,6-dimethylbenzimidazole and some yellow degradation products. It was also noted that any analogues present could be separated (after removal of red acids on a column of basic cellulose) by paper chromatography with a wet s-butanol - acetic acid system in the presence of hydrogen cyanide vapour,[2] but that direct paper chromatography of hydroxocobalamin as such gave slow-running diffuse zones. Further work has shown that the preliminary removal of red acids is not necessary, provided that hydroxocobalamin is first converted to the cyano-form, and that paper chromatography is suitable for determining impurities in hydroxocobalamin and for the assay of hydroxocobalamin injection products. The proposed method is more precise and yields more information than microbiological assay, whether this is carried out on the hydroxo- or the cyano-form.

There is, however, a resulting disadvantage from converting to the cyano-form for paper chromatography, in that it is not then possible to detect the presence of any cyanocobalamin in the sample. For solid samples of hydroxocobalamin this is of no consequence, as cyanocobalamin is measured, together with any other neutral cobalamin, by column chromatography on basic and acidic celluloses.[1]

However, for solutions, particularly those of unknown origin when no corresponding solid can be tested and samples are small, column chromatography is not particularly suitable and preliminary extraction with phenol[2] must first be carried out to remove electrolytes. For either solid or solutions the ratio of the optical density at 361 mμ to that at 351 mμ, determined on a suitable dilution, provides a simple means of ensuring that the sample is substantially free from cyanocobalamin. This ratio for mixtures of hydroxo- and cyanocobalamin in different proportions is shown below—

Hydroxocobalamin, % ..	100	95	90	85	80	0
Ratio, E_{361}/E_{351}	0·65	0·69	0·72	0·76	0·79	1·60

Although some variations in the ratio for authentic samples of hydroxocobalamin are to be expected, it would appear that limits of 0·63 to 0·69 would exclude more than about 5 per cent. of cyanocobalamin.

METHOD

DETERMINATION OF COLOURED IMPURITIES IN HYDROXOCOBALAMIN—

Shake 1 litre of a mixture of equal parts of water and s-butanol, allow it to separate overnight at a constant temperature between 25° and 30° C, and transfer the lower layer to the bottom of a paper-chromatography tank with the addition of 1 per cent. of acetic acid and 0·5 g of potassium cyanide.

Dissolve about 2 mg of sample, accurately weighed, in 0·5 to 1·0 ml of water, and add 1 drop only of freshly prepared 0·3 per cent. aqueous hydrocyanic acid. After it has been mixed, set the solution aside for 15 minutes, and then place on a filter-paper (Whatman 3 MM paper, 20 inches × 9 inches) in a narrow line 4 inches from one end, leaving 1 inch clear at both sides. Dry each superimposed streak in a stream of nitrogen and without applying heat. Serrate the lower edge, suspend from a trough in the paper-chromatography tank, and allow the paper to equilibrate overnight at the same temperature as before.

Add 5 per cent. of s-butanol and 1 per cent. of acetic acid to the upper layer, and fill the trough with this liquid. Allow the chromatogram to run until the cyanocobalamin has travelled about two thirds of the length of the paper, transfer to a fume cupboard, remove the paper, and dry in a current of air. Cut out and discard the main cyanocobalamin band, and from both remaining areas of paper trim off and discard any uncoloured top and bottom zones. Form both strips of paper into cylinders by fastening the vertical edges of each together, and stand them on wet filter-paper so that the coloured material is eluted to the top edges. Unfasten and trim off the uncoloured paper, and elute the coloured impurities by descending-solvent chromatography with the minimum amount of water. Combine the eluates, and dilute to a convenient volume with water (preferably not more than 25 ml). Filter through sintered glass, and measure the optical density at the maximum near 361 mμ in a 2-cm cell. Repeat the procedure on paper similar in size and position, cut from a blank chromatogram, and deduct the optical density observed at the same wavelength.

Calculate the coloured impurities ($E^{1\%}_{1\text{cm}} = 207$) as a percentage of the dry solid.

In the experimental work on which this method is based, it became clear that certain precautions are necessary if accurate results are to be obtained. The addition of the initial cyanide is critical; if the normal procedure via the dicyanide is carried out (addition of potassium cyanide in alkaline solution and then acidification with acetic acid) extra bands appear in the chromatogram—in particular, a strongly adsorbed red band remains at the origin. Hydrocyanic acid solution is the most convenient alternative. The amount and concentration must be carefully controlled, as even moderate excess can cause formation of red impurities. Under these conditions, conversion of the hydroxocobalamin to the cyanoform is only about 90 per cent. complete, the remainder being converted by the cyanide in the atmosphere of the chromatography tank.

A sample of hydroxocobalamin (approximately 5 mg) was purified by paper chromatography in a cyanide-free atmosphere. The main hydroxocobalamin band was then eluted and re-run in an acidic cyanide tank without adding hydrocyanic acid to the solution before streaking on to paper. Only the main cyanocobalamin band was detected.

Although complete conversion to cyanocobalamin can be effected during equilibration in the tank, it is desirable to minimise, by adding cyanide to the sample solution, the main losses that may occur with hydroxocobalamin during the loading of the paper. This preliminary conversion is essential before applying the phenol extraction if the sample contains electrolytes (see below).

The most likely source of cyanocobalamin loss is irreversible adsorption on the paper. The recoveries shown below are calculated as a percentage of the initial optical density at 361 mμ. For each test 30 to 60 mg were dissolved in 10 ml of water, and a portion (0·4 to 0·6 ml) was streaked on to paper. A similar portion was diluted to 100 ml, and the optical density at 361 mμ was measured; the results were—

Dry sample taken for chromatogram, mg	3·37	1·55	2·33	2·35	2·35	2·37
Recovery, %	100	101	98	98	99	96

These figures, obtained under favourable conditions with considerable attention to detail, show that the recovery is not reliably 100 per cent. (this is hardly to be expected with paper chromatography), and under routine conditions a recovery of less than 95 per cent. may occasionally be recorded.

The losses of impurities would not, however, be important if they were proportional to those of cyanocobalamin. Two tests were therefore made to show that, with impurities of the amount and type normally found, the losses were, in fact, proportional.

A chromatogram was produced from a small amount of monobasic red acids (equivalent to a few per cent. in a 5-mg sample of cyanocobalamin) and another from a vitamin B_{12} analogue containing benzimidazole as the nucleotide base.

The results in Table I were calculated by comparing the optical densities at about 361 mμ of solution before and after chromatography. During these tests it was found that any paper of the developed chromatogram showing no pink colour when examined wet against the light could safely be discarded. Blank values determined on the paper were found to be about 1 per cent., provided that the paper was not old or discoloured.

TABLE I

THE RECOVERY OF RED ACIDS AND BENZIMIDAZOLE FROM CHROMATOGRAMS

Sample	Weight of sample chromatographed, mg	Recovery, %
Monobasic "red acids"	0·096	103
"Benzimidazole" analogue of cyanocobalamin	0·19	99

The method of expressing results for coloured impurities requires comment. The cyano-compounds separated have maxima at about 360 mμ, but although an $E^{1\%}_{1\,\text{cm}}$ of 204 has been reported[3] for pseudo-vitamin B_{12} and for factor A, and a sample of red acids examined in this laboratory had an $E^{1\%}_{1\,\text{cm}}$ of approximately 195 at 361 mμ, the mean $E^{1\%}_{1\,\text{cm}}$ at 361 mμ cannot be known precisely.

It is necessary for the purpose of the test, therefore, to define the coloured impurities as the ones separable in their cyano-forms by the method described, and then to specify an $E^{1\%}_{1\,\text{cm}}$ value for the calculation. The adoption of 207 has been shown to be unlikely to introduce any substantial error. Results are then conveniently expressed as a percentage of the dry solid taken.

Examination of commercial hydroxocobalamin from three different sources by this method indicated the presence of 0·5 to 6·4 per cent. of coloured impurities, the majority lying between 2 and 4 per cent.

DETERMINATION OF HYDROXOCOBALAMIN IN INJECTION PRODUCTS—

It is normally necessary to extract hydroxocobalamin from preparations before it can be chromatographed, as any electrolyte present will interfere with the separation, and other ingredients also may be eluted from the paper and interfere spectrophotometrically. Preliminary experiments had shown that hydroxocobalamin was sensitive to heat and, to some extent, to light and that degradation was less likely to occur if, before extraction, it were converted to the cyano-form. Moreover, it is much easier to obtain a clean precipitation with ether in the presence of cyanide.

Extraction procedure—Place in a 50-ml separating funnel an accurately measured amount of the sample containing not less than 2 mg and not more than 5 mg of hydroxocobalamin. Dilute, if necessary, to 5 ml with water. For each 2 ml of solution add 1 drop of freshly prepared 0·3 per cent. w/v aqueous hydrocyanic acid, mix, and set aside for 15 minutes. Extract with 2 ml of a 50 per cent. solution of phenol in chloroform. Repeat the extraction with 1-ml portions of phenol - chloroform mixture until no more colour is extracted. Collect all the extracts in a 100-ml centrifuge tube, and add 15 ml of acetone and 80 ml of anaesthetic ether. Mix, and spin in a centrifuge at 1500 *g* for 10 minutes. Decant the colourless ether layer, mix the residue with a further 50 ml of ether, spin in a centrifuge, and again decant. Repeat the washing of the residue with ether, until no odour of phenol remains after the ether has been allowed to evaporate from the residue. Dissolve the residue in the minimum amount of water, and transfer the solution quantitatively to paper for chromatography and subsequent determination of coloured impurities as described in the procedure for hydroxocobalamin. Measure the optical density at the maximum near 351 mμ on a suitable dilution of the sample, and calculate the apparent hydroxocobalamin in the sample; use $E^{1\%}_{1\,\text{cm}} = 190$. Deduct from the value obtained the proportion of coloured impurities found, and report the results as micrograms of hydroxocobalamin per millilitre. The ratio of the optical density at 361 mμ to that at 351 mμ should also be determined on the original solution, to permit calculation of the amount of any cyanocobalamin in the sample.

The amount of phenol - chloroform mixture used for extraction should be as small as possible, so as to minimise the volume of ether required for precipitation. This is preferable to re-extraction into water, as the cyanocobalamin is obtained solid and no further concentration step is needed.

As a check on possible losses during the extraction, the hydroxocobalamin in 50 ml of a solution containing 1000 μg per ml was partially degraded by steaming for 4 hours. One 5-ml portion to which two drops of 0·3 per cent. w/v hydrocyanic acid had been added was set aside for 15 minutes and was then loaded on to paper for determining coloured impurities. Another 5-ml portion was treated with hydrocyanic acid in the same way, but was then extracted with phenol - chloroform mixture before chromatography. Duplicate results for coloured impurities were 17 and 16 per cent. without and 17 and 18 per cent. with the extraction step. Before it was steamed the hydroxocobalamin contained 4 per cent. of coloured impurities.

Consideration of possible impurities in injection solutions suggests that the coloured impurities determined by the above method may provide a good indication of any deterioration. Although the small amounts of analogues and other cobalamins present (even if less stable than the hydroxocobalamin) are unlikely to affect substantially the total amount of impurities occurring after storage, the amount of red acids increases, and the total coloured impurities increase by the same amount. Provided that analogues and interfering substances are present in negligible amounts, determination of the coloured impurities permits correction of the optical density at 351 mμ and an accurate determination of hydroxocobalamin.

To check this, 100-ml portions of an aqueous solution of hydroxocobalamin (1000 μg per ml) were steamed for different times to produce a substantial degree of breakdown. These solutions were assayed microbiologically (three consecutive daily tests, in duplicate, at two levels with *Escherichia coli* M200); the optical density at 351 mμ was measured, and the coloured impurities were determined. The results are shown in Fig. 1. It will be noted that for heat-induced breakdown the drop in optical density is approximately half the drop in microbiological potency, and the differences between the two sets of values are well correlated with the amounts of coloured impurities. The figures for the solution that has not been treated in an autoclave are anomalous in that the microbiological assay results (fiducial limits for a single day's assay, 2 operators at 2 dose-levels, are ±11 per cent., P = 0·95) are higher than the corresponding ultraviolet figure, although absorbing impurities are present. The hydroxocobalamin sample chosen, however, was not of high purity and *E. coli* M200 responds to a wide range of cobalamin-like substances of different stabilities and with different effects on zone size. Factor B, for example, produces a large rather diffuse zone in comparison with the same amount of hydroxocobalamin. The higher microbiological results obtained may be

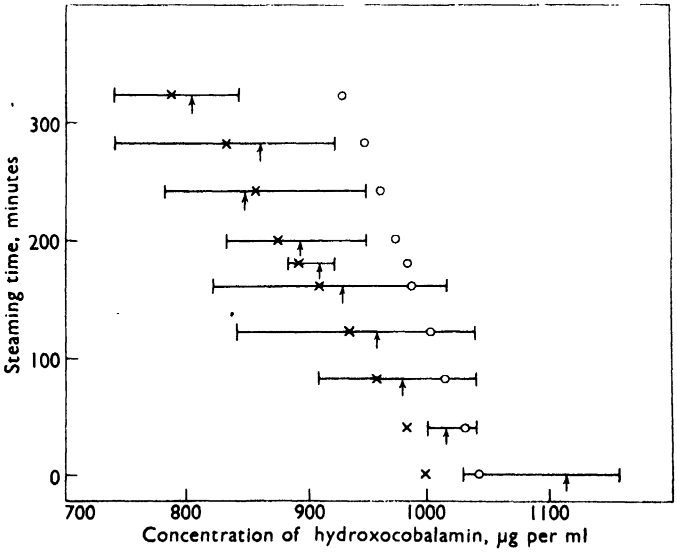

Fig. 1. Effect of steaming time on break-down of hydroxocobalamin: ○, ultraviolet spectrophotometric results; ×, ultraviolet spectrophotometric results *minus* red impurities; ——, microbiological results (range over 3 days); ↑, mean of biological results

attributed to the presence of small amounts of such compounds, having comparatively low stability.

It has not so far been possible to prove that solutions of hydroxocobalamin for injection (100 to 1000 µg per ml) stored at ambient temperatures undergo the same type of breakdown, but we have found that the increase in coloured impurities in typical preparations is less than 1 per cent. at room temperature and less than 2 per cent. at 31° C over a period of 7 months.

It is suggested that a limit of not more than 5 per cent. for total coloured impurities determined by the proposed method should be attainable for hydroxocobalamin; a further 10 per cent. may be considered permissible for their development during manufacture and later storage, provided that this is offset by an adequate overage of hydroxocobalamin itself.

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The Determination of 4-Methyl-2,6-di-t-butyl Phenol in Liquid Paraffin B.P.

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A colorimetric procedure is described for determining the antioxidant 4-methyl-2,6-di-t-butyl phenol at the 10 p.p.m. level in liquid paraffin B.P. The antioxidant is converted to the intensely coloured 3,5,3′,5′-tetra-t-butyl stilbene-4,4′-quinone by oxidation with lead dioxide in isopropanol azeotrope. The quinone is extracted into cyclohexane, and the optical density is measured at 420 mμ. A sample of uninhibited liquid paraffin is necessary, but, for the instances when this is not available, a method is described for extracting the antioxidant and recovering the liquid paraffin.

LIQUID paraffin B.P. is a mixture of hydrocarbons derived from petroleum, which, under the present requirements of the British Pharmacopoeia, may be stabilised by the addition of not more than 10 p.p.m. of α-tocopherol. From 1963 onwards, however, it is proposed to permit the additional use of up to 10 p.p.m. of 4-methyl-2,6-di-t-butyl phenol (4M26B), and as a result of this a request was received from the B.P. commission for a suitable method of determining 4M26B in the presence of α-tocopherol.

The general problem of determining low concentrations of antioxidants in the wide range of materials to which they are now added poses two difficult analytical problems—separation of the additive and development of sensitive and preferably specific methods for its determination.

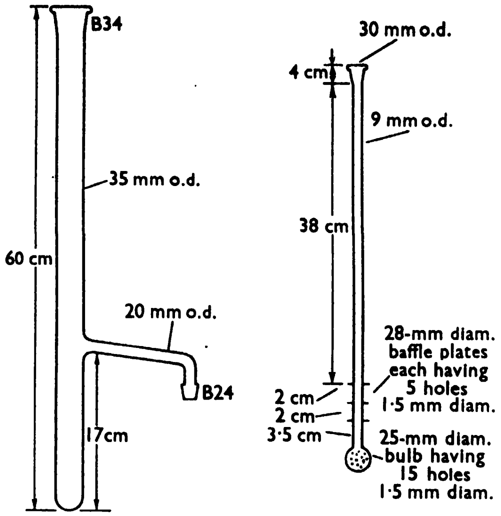
Among the procedures for separating antioxidants reported in the literature in recent years are extraction,[1,2] steam-distillation,[3,4,5,6] column chromatography[7] and gas - liquid chromatography.[8,9] In our hands each of these procedures has given satisfactory results for particular types of inhibited materials, but none proved satisfactory for liquid paraffin, and a novel extraction procedure was eventually adopted. Methods for determining 4M26B have been based mainly on measurement of ultraviolet absorption[10,11] or colorimetry,[3,6,12] but the variable background absorption of liquid paraffin ruled out ultraviolet measurement, and the colorimetric methods lacked sensitivity or specificity or both.

For the work described here it was decided to investigate the oxidation of 4M26B with lead dioxide as the basis of a colorimetric method. An effective separation procedure for removing 4M26B from liquid paraffin was simultaneously developed, but was ultimately not required owing to the high sensitivity of the final colorimetric method.

EXPERIMENTAL

EXTRACTION OF 4-METHYL-2,6-DI-t-BUTYL PHENOL—

Although continuous liquid extraction has been used with success for separating 4M26B from several solid materials, little has been reported about the analogous use of continuous liquid - liquid extraction. It was therefore decided to explore this possibility by using the apparatus shown in Fig. 1. A survey of common organic solvents, in which 4M26B is soluble and liquid paraffin virtually insoluble, showed that lower alcohols should be the most suitable extractants. Preliminary experiments with ethanol and methanol on a sample of liquid paraffin containing 20 mg of 4M26B per 100 ml gave recoveries of only 70 to 80 per cent., and observations of the operation of the liquid - liquid extractor suggested that poor mixing, owing to the high viscosity of the liquid paraffin, might well be the cause of these low recoveries. The same sample of liquid paraffin was therefore diluted with cyclohexane and again extracted with methanol for 6 hours. Recoveries of 96·7 and 98·0 per cent. were then achieved. When applied to a sample of liquid paraffin containing 10 p.p.m. of 4M26B, complete extraction was again achieved.


B34
35 mm o.d.
60 cm
20 mm o.d.
B24
17cm
30 mm o.d.
4 cm
9 mm o.d.
38 cm
28-mm diam. baffle plates each having 5 holes 1·5 mm diam.
2 cm
2 cm
3·5 cm
25-mm diam. bulb having 15 holes 1·5 mm diam.

(*a*) (*b*)

Fig. 1. Continuous liquid extraction apparatus: (*a*) extractor; (*b*) solvent diffuser

The measurement of ultraviolet absorption, although satisfactory for the extraction experiments, could not, however, be adopted for the final method. Liquid paraffin exhibits variations in absorption from batch to batch, thus making it necessary to use in the blank test the identical base oil present in material under test. Since this is impracticable in a routine method, attention was turned to the development of a more generally applicable colorimetric procedure. The method ultimately developed proved so sensitive that removal of 4M26B by continuous extraction was unnecessary, and, although a blank test was still required, any liquid paraffin free from 4M26B was suitable. However, when uninhibited liquid paraffin is not available or when any doubt exists, the extraction procedure provides a convenient way of preparing liquid paraffin suitable for the blank test, and for this reason full experimental details are given below.

EXTRACTION PROCEDURE—

Measure 90 ml of liquid paraffin B.P. into a 250-ml beaker, and add 30 ml of cyclohexane; mix well, and then transfer to the liquid - liquid extractor. Introduce the solvent diffuser, and fit a double-surface water condenser to the top of the extractor. The liquid level in the extractor should be about 2 cm below the bottom of the side-arm. Connect the side-arm to a round-bottomed 250-ml flask containing 100 ml of analytical-reagent grade methanol, and place in a suitably sized heating mantle (alternatively, a 250-ml conical flask and a hot-plate may be used). Bring the contents of the flask to the boil, and extract the liquid paraffin - cyclohexane mixture for 6 hours. Cool to room temperature, and then transfer the contents of the extractor to a 250-ml separating funnel. Allow the layers to separate, and then run off the liquid paraffin - cyclohexane layer into a round-bottomed 250-ml flask. Connect the flask to the vacuum-distillation apparatus, and distil off all the cyclohexane at a pressure not exceeding 10 mm of mercury.

Cool the contents of the boiler, and store in a clean bottle until required.

DEVELOPMENT OF COLORIMETRIC METHOD—

When an alcoholic solution of 4M26B is boiled under reflux with lead dioxide, a yellow colour is formed that has an absorption maximum at 420 mμ. Attempts to carry out the reaction directly on a solution of 4M26B in liquid paraffin, or on liquid paraffin diluted with cyclohexane, did not, however, produce a colour. Addition of the latter alone to an ethanolic solution of the antioxidant also resulted in considerable diminution of the colour, thereby demonstrating that the hydrocarbons have this common property. All experiments on the colour reaction were carried out in 250-ml conical flasks fitted with double-surface water condensers. The flasks and contents were heated on a hot-plate for the appropriate period, and then cooled to room temperature. After filtration through a No. 4 sintered Gooch crucible, the solutions were made up to a suitable volume, and the optical densities were measured at 420 mμ.

EFFECT OF AMOUNT OF LEAD DIOXIDE USED—

Different weights of lead dioxide in the range 0·1 to 2·0 g were boiled under reflux for 3 hours with 75-ml portions of a methanol solution containing 2 mg of 4M26B per 100 ml. Within the limits of experimental error, optical densities were identical. On the grounds of economy of reagents, 0·1 g of lead dioxide was adopted as standard for all subsequent work in which methanol was used. Subsequently, however, when isopropanol azeotrope was found to be a more suitable solvent, it was necessary to increase this to 1 g in order to obtain reproducible colours.

EFFECT OF TIME—

Portions (75 ml) of the same methanol solution as above were boiled under reflux for different periods up to 4 hours, and then diluted to 100 ml with methanol. The results were—

Time, hours	½	1	1½	2	3	4
Optical density (1-cm cell) . .	0·492	0·588	0·555	0·555	0·545	0·505

Blank experiments carried out at the same time did not produce any perceptible colours.

Later it was realised that addition of water resulted in a considerable enhancement in the intensity of the colour, and the work was repeated with 78 per cent. v/v aqueous methanol containing 1 mg of 4M26B per 100 ml. Similar experiments were carried out with isopropanol azeotrope as solvent. In each test the solutions were diluted to 100 ml and the optical densities measured in ½-cm cells. The results were—

Time, hours		½	1	1½	2	2½	3	4
Optical density (½-cm cell)	Aqueous methanol . .	0·282	0·348	0·435	0·460	0·475	0·473	0·475
	Isopropanol azeotrope	0·013	0·156	0·284	0·322	0·358	0·355	0·365

As a result of these experiments a standard reaction time of 3 hours was adopted.

EFFECT OF WATER—

Experiments were carried out to determine the effect of adding water to the reaction mixture. In each test 1 mg of 4M26B was boiled under reflux with 90 ml of aqueous methanol or isopropanol containing various proportions of water. The solutions were diluted to 100 ml before measurement. The results were—

Ratio of alcohol to water		85/5	80/10	70/20	60/30	45/45	30/60	20/70
Optical density (½-cm cell)	Methanol	0·263	0·350	0·448	0·225	0·258	—	—
	Isopropanol	0·228	0·315	0·475	0·552	0·594	0·495	0·025

These results show that water considerably enhances the intensity of the developed colours. At the optimum ratio of 70 parts of methanol to 20 parts of water (78 per cent. v/v of ethanol) the optical density is almost twice that obtained when no water is present. With isopropanol the optimum ratio is 45/45, but, because of solubility difficulties, isopropanol azeotrope was adopted for subsequent work.

EFFECT OF α-TOCOPHEROL—

α-Tocopherol does not produce a colour when oxidised with lead dioxide in methanol or isopropanol solution.

EXTRACTION OF COLOUR INTO CYCLOHEXANE—

During attempts to apply the colour reaction to methanol extracts of 4M26B, it was observed that the developed colour partitioned between the alcohol and the small amount of liquid paraffin that separated out on cooling. This suggested that considerable concentration of the intensity of the colour would be achieved by extraction into a hydrocarbon solvent after development of the colour.

Portions of liquid paraffin (5 ml) containing 0·5, 1·0 and 1·5 mg of 4M26B were boiled under reflux for 3 hours with 100 ml of isopropanol azeotrope and 1 g of lead dioxide. After the solutions had been cooled they were diluted with 100 ml of water, and then extracted 3 times with 10-ml portions of cyclohexane, which at this stage does not affect the colour reaction. The bulked extracts were filtered into 50-ml calibrated flasks, made up to volume with cyclohexane, and the optical densities measured at 420 mμ. Results of these determinations are shown in Fig. 2 and indicate that a considerable increase in sensitivity had in fact been achieved.

Although these results show good repeatability, it will be seen that the best straight line fitting these points does not pass through the origin. This effect was noticed in all subsequent work, and it was concluded that there is a certain minimum concentration of 4M26B (about 0·2 mg) below which the Beer - Lambert law is not obeyed. This difficulty was obviated in the final form of the method by the addition of known amounts of 4M26B to samples, to increase the minimum concentration above the 0·2-mg level.

DIRECT COLORIMETRIC DETERMINATION—

From the initial experiments it was known that liquid paraffin had a deleterious effect on the colour reaction. It was considered, however, that this could be more than counterbalanced by the increase in sensitivity gained by extracting the colour into cyclohexane. In experiments to prove this point, isopropanol azeotrope was chosen as solvent, since liquid paraffin is more soluble in this solvent than in methanol and it provides a convenient way of adding water necessary for the reaction. Various amounts of liquid paraffin were added to 100-ml portions of isopropanol azeotrope containing 1 mg of 4M26B, 1 g of lead dioxide was added, and the mixture was boiled under reflux for 3 hours. After dilution with water, the colour was extracted into 50 ml of cyclohexane, and the optical density was measured. The results shown below indicate that the reduction in optical density produced by liquid paraffin is directly proportional to the amount present.

Volume of liquid paraffin, ml	0	5	10	20
Optical density (½-cm cell)	0·805	0·695	0·595	0·419

By comparison with the results showing the effect of water, p. 299, it can be seen that, even in the presence of 10 ml of liquid paraffin, the optical density for 1 mg of 4M26B shows an increase of about 30 per cent. over the maximum value obtained with aqueous methanol. It was decided, therefore, to adopt a sample size of 10 ml as standard for a liquid paraffin containing 10 p.p.m. of 4M26B.

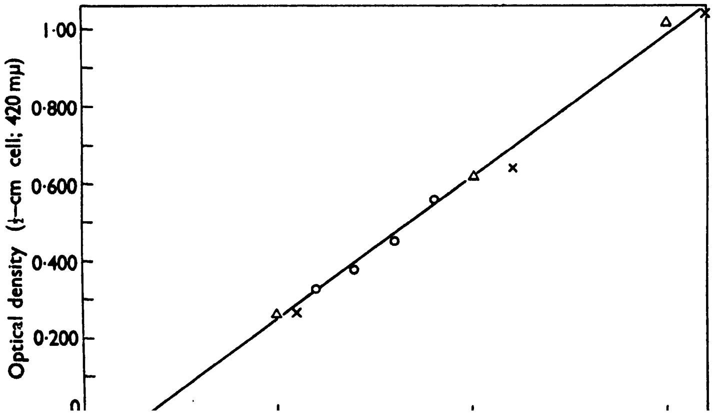


Fig. 2. Determination of 4M26B in liquid paraffin by extraction of the colour into cyclohexane

METHOD

APPARATUS—

Spectrophotometer—A Unicam SP500, or any suitable instrument capable of measuring optical density at 420 mμ, and 1-cm glass or silica cells.

REAGENTS—

Lead dioxide.

Isopropanol.

Isopropanol azeotrope—Dilute 85 parts of isopropanol with 15 parts of distilled water, and mix well.

Cyclohexane.

Standard solution of 4-methyl-2,6-di-t-butyl phenol—Dissolve 0·1000 ± 0·0005 g of pure 4M26B in 50 ml of analytical-reagent grade methanol contained in a 100-ml calibrated flask. Make up to the mark with analytical-reagent grade methanol, and mix well. Dilute 10 ml of this solution to 100 ml with analytical-reagent grade methanol, and mix well.

1 ml ≡ 0·1 mg of 4M26B.

PROCEDURE—

In each of four 500-ml conical flasks place exactly 10 ml of the liquid paraffin under test and then 0, 2, 3 or 4 ml of the standard solution of 4M26B (equivalent to 0, 0·2, 0·3 and 0·4 mg of 4M26B). In a further four identical flasks place 10-ml portions of "blank" liquid paraffin (*i.e.*, one containing α-tocopherol, but no 4M26B—see Note 1) and then 0, 2, 3 or 4 ml of the standard solution of 4M26B. Add to the contents of all the flasks 2 g of lead dioxide, 200 ml of isopropanol azeotrope and a few boiling-beads. Connect the flasks to double-surface water condensers, and boil under reflux for 3 hours on a hot-plate. At the end of this period cool to room temperature, and then quantitatively transfer the contents of each flask in turn to 750-ml separating funnels. Add 300 ml of distilled water to the contents of each funnel, and then extract the colours with three 10-ml portions of cyclohexane. Filter the extracts through Whatman No. 42 filter-papers into 50-ml calibrated flasks. Add exactly 10 ml of isopropanol to the contents of each, and then make up to the mark with cyclohexane, and mix well. Measure the optical density in 1-cm cells of each solution at 420 mμ against cyclohexane. Plot graphs relating optical density to milligrams of 4M26B added for both sample and blank liquid paraffins (see Fig. 3).

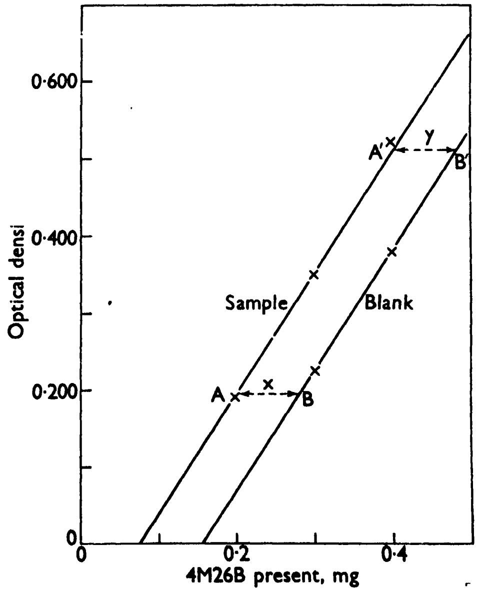


Fig. 3. Determination of 4M26B in a liquid paraffin containing 8 p.p.m. of 4M26B

Concentration of 4M26B, p.p.m. = 100 x
or = 100 y

CALCULATION—

Let the point on the sample graph corresponding to 0·2 mg of added 4M26B be A and that corresponding to 0·4 mg of added 4M26B be A′.

Draw lines parallel to the X axis from A and A′ to intersect the "blank" graph at B and B′.

Let the number of milligrams of 4M26B corresponding to AB and A′B′ be x and y, respectively (x and y will normally be identical; if they differ by more than 0·01 mg, repeat the determination).

Then—

$$\text{Concentration of 4M26B in sample, p.p.m.} = 100\,x$$
$$\text{or} = 100\,y$$

NOTE—

1. If a blank liquid paraffin (*i.e.*, one containing no 4M26B) is not available, a suitable blank can be prepared by the extraction procedure described on p. 298.

RESULTS AND DISCUSSION

Some results on factitious mixtures of 4M26B in liquid paraffin were—

4M26B added, p.p.m.	6	10	12	15	20
4M26B found, p.p.m.	6,6	8, 9, 9	12, 11, 12	13, 14, 15	18, 18

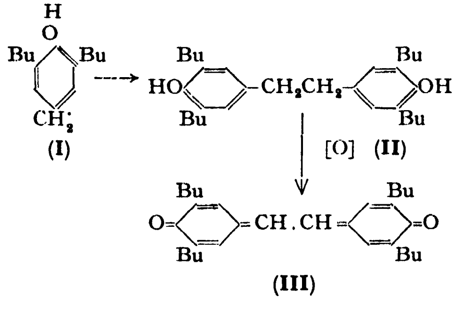
These results demonstrate that the method is sufficiently precise for determining 4-methyl-2,6-di-t-butyl phenol in liquid paraffin.

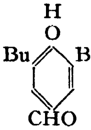
DISCUSSION—

Although the mechanism of the colour reaction is not fully understood, it seems certain that the main product of the oxidation is 2,5,3′,5′-tetra-t-butylstilbene-4,4′-quinone (**III**), which is a brilliant red and responsible for the colour produced.

Cosgrove and Waters[13] have reported the formation of **III** by the action of benzoyl peroxide on 4M26B, and suggest that this product is formed via a 3,5-di-t-butyl-4-hydroxybenzyl radical (**I**). This view is supported by the fact that, in our work, the oxidation of 4M26B in methanol with lead dioxide nearly always gave an initial blue colour before development of the yellow. Cook[14] also confirms this view, and suggests that an expected intermediate oxidation product would be **II**.

The probable reaction is therefore—





(IV)

Bu = t-Butyl.

Cook found that the oxidation of 4M26B with limited amounts of oxygen, lead dioxide, alkaline ferricyanide or hydrogen peroxide always gave mixtures of **II** and **III**. He isolated **II** from the lead dioxide oxidation of 4M26B in diethyl ether as a light yellow powder (m.p. 171° to 175° C), which, on recrystallisation from alcohol, gave white needles (m.p. 174° to 175° C). **III** was obtained, by concentration of the mother liquor, as bright red crystals (m.p. 296° to 301° C; 314° to 315° C after recrystallisation).

Wasson and Smith[15] have reported that oxidation of 4M26B with chromic anhydride-acetic acid gives a white aldehyde (m.p. 185° C) believed to be 3,5-di-t-butyl-4-hydroxybenzaldehyde (**IV**). Cook does not report finding any trace of this. We have shown, however, that if the oxidation of 4M26B with lead dioxide is carried out in methanol, and more especially if water is also present, the aldehyde **IV** is indeed formed.

One gram of 4M26B was oxidised with 3 g of lead dioxide in 120 ml of analytical-reagent grade methanol. A copious white precipitate was formed, which proved to be lead hydroxide (lead found, 85·9 per cent.; theory, 86·0 per cent.). Dilution of the red methanol filtrate with water gave a yellow powder (m.p. 172° to 176° C) after one recrystallisation from ethanol. This material had a C=O value of 6·3 per cent. (theory for **IV**, 11·9 per cent.) and was probably a mixture of **II** and **IV**. Cook quotes a mixed melting-point for this mixture of 174° C. Concentration of the mother liquor gave dark red crystals (m.p. 300° to 304° C); this was the quinone **III**.

In a second similar experiment the oxidation was carried out in the presence of 25 ml of water. Dilution of the filtrate in the manner described above yielded a pale yellow precipitate (m.p. 182° to 184° C). After sublimation, this material was isolated as an almost white powder (m.p. 185° to 188° C, C=78·0 per cent., H=9·7 per cent., m.p. of 24DNP derivative 239° to 241° C; theory, m.p. 189° C, C=77·2 per cent., H=9·4 per cent., m.p. of 24DNP 236° C). This material was 3,5-di-t-butyl-4-hydroxybenzaldehyde (**IV**). Concentration of the mother liquor again gave the red quinone **III** (m.p. 304° to 308° C, C=82·9 per cent., H=9·6 per cent.; theory, C=83·1 per cent., H=9·7 per cent.).

Cook's preparation of **II** and **III** by oxidation of 4M26B in diethyl ether with lead dioxide was next duplicated. **II** was isolated as a dark yellow powder (m.p. 169° to 172°C, C=81·9 per cent., H=10·7 per cent.) and **III** was obtained as scarlet red crystals (m.p. 315° to 319° C, C=82·3 per cent., H=9·6 per cent.). No trace of aldehyde was found. It is evident, therefore, that although the final product of oxidation is the stilbene quinone (**III**) in each instance, the intermediate oxidation products are dependent on the solvent in which the reaction is carried out.

We thank Mrs. L. F. C. Underwood for her valuable assistance with the experimental work.

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The Determination of the Total Sulphur Dioxide Content of Ciders

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The factors involved in determining total sulphur dioxide in alcoholic beverages are discussed, and an iodimetric method is described in which reflux distillation from dilute orthophosphoric acid and a carrier-stream of nitrogen are used. The reflux condenser effectively retains other iodine-reducing substances, and the iodimetric titration eliminates interference due to the presence of acetaldehyde.

In principle, the determination of sulphur dioxide in a foodstuff requires the distillation of the sample with acid and collection of the sulphur dioxide in some suitable solution in which it can be determined. The fact that so many methods and modifications of conditions of distillation and determination have been proposed[1,2,3] indicates that no entirely satisfactory general procedure has yet been devised. The extensive literature on the determination of sulphur dioxide is confused and sometimes contradictory, possibly owing to lack of appreciation of the errors involved in a particular method, especially if it is applied to a product for which it is not entirely suitable. In most methods there are several possible sources of error, some causing high results and some low, so that apparently correct results can be obtained fortuitously by combination of these effects. Unless, therefore, the separate errors are recognised and each minimised or evaluated, even the apparent complete recovery of added sulphur dioxide is no guarantee of the general reliability of a method. Further, different foodstuffs present different problems owing to the presence of interfering substances.

Any method for the accurate determination of sulphur dioxide in alcoholic beverages should take account of (*a*) the presence of volatile substances other than sulphur dioxide that will give appreciable blank values and (*b*) the presence of acetaldehyde, which can react with sulphur dioxide before it is oxidised (with hydrogen peroxide or iodine) in the receiver. This latter requirement is frequently overlooked; the error involved is usually small (1 to 2 per cent.), but cannot be neglected in an accurate determination.

The various methods for determining sulphur dioxide in wines have been fully reviewed by Deibner.[3] Apart from Ripper's method[4,5] of direct titration with iodine, which is well known to be subject to errors, the most commonly used methods all involve distillation and can be divided into two main groups.

> *Alkalimetric methods*—The acidified sample is boiled under reflux and the sulphur dioxide carried over in a stream of air or inert gas into neutralised hydrogen peroxide, where it is oxidised to sulphuric acid and, finally, titrated with sodium hydroxide. The result can be checked gravimetrically by precipitation as barium sulphate. This is essentially Monier Williams' method.[6] The presence of volatile acids in the sample may lead to high results by titration (depending on the efficiency of the reflux condenser), but does not affect results determined gravimetrically.
>
> *Iodimetric methods*—The sulphur dioxide distilled from the acidified sample is collected in a suitable receiving solution and subsequently titrated with iodine. Various receiving solutions have been proposed, *e.g.*, sodium hydroxide,[3] sulphuric acid,[7] and iodine.[8] The advantage of this method is the convenience and sensitivity of the iodimetric titration.

Two methods for determining total sulphur dioxide in wines have been described recently, one alkalimetric and the other iodimetric. In describing the first, Kielhöfer and Aumann[9] discussed possible sources of error in the iodimetric titration of sulphur dioxide and concluded that Paul's alkalimetric method is preferable; Paul later described his method in more detail.[10] The wine (10 or 20 ml) is boiled under reflux with 5 ml of 25 per cent. phosphoric acid for 15 minutes in a Lieb - Zacherl apparatus[11]; the liberated sulphur dioxide is carried over in an air-stream into 3 ml of 0·3 per cent. hydrogen peroxide (previously neutralised to Tashiro indicator) and titrated with 0·01 N sodium hydroxide. The essential feature of this apparatus is a highly efficient reflux condenser. Blank determinations of any volatile acids not retained by the condenser can be made by adding a trace of hydrogen peroxide to the sample before distillation. Paul, however, claims that blank determinations for normal wines are negligible, and this was confirmed by Kielhöfer and Aumann. Although this method is satisfactory from the point of view of the blank value, it cannot allow for the error due to acetaldehyde.

In the second method, described by Diemair, Koch and Hess,[12] the sample is distilled from dilute phosphoric acid, and the distillate is collected in buffer at pH 3·4 to prevent loss of sulphur dioxide by volatilisation. The distillate is titrated with 0·01 N iodine, first at pH 3·4 to oxidise the free sulphur dioxide and then after solid sodium hydrogen carbonate has been added to dissociate any acetaldehyde bisulphite. Although this method caters for the presence of acetaldehyde, it makes no allowance for the blank value of volatile iodine-reducing substances other than sulphur dioxide. With ciders this blank value has been found to represent about 5 p.p.m. of sulphur dioxide.

The proposed method combines the advantages of both the procedures described above, since it gives zero blank corrections with ciders and complete recovery of sulphur dioxide in the presence of acetaldehyde.

METHOD

GENERAL PRINCIPLES—

The development of this method was based on the considerations listed below:—

1. In order to be able to titrate acetaldehyde bisulphite it is essential to use an iodimetric method, since the adjustment of pH necessary to dissociate the bound sulphur dioxide is not permissible in the alkalimetric procedure.

2. In principle, Diemair, Koch and Hess's method[12] appeared to offer a rapid and convenient means of determining total sulphur dioxide, particularly if it could be done by steam-distillation in a Markham apparatus.[13] Recovery of sulphur dioxide (10 ml at 300 p.p.m.) from acetaldehyde bisulphite was 99·5 per cent. by this procedure. When, however, ciders that were shown to be completely free from sulphur dioxide (by acid distillation and colorimetric determination[14] by pararosaniline and formaldehyde) were analysed by this method, the cider blank value, due to volatile iodine-reducing substances, corresponded to 3 to 8 p.p.m. of sulphur dioxide. No means could be found of reducing the magnitude of this blank value. The alternative, of determining the blank value by adding a trace of hydrogen peroxide to the cider to oxidise the sulphur dioxide before distillation, was tested on a sulphur dioxide free cider. This was unsuccessful since the blank value was much greater (18 p.p.m. as sulphur dioxide) after treatment with hydrogen peroxide than without (3 p.p.m.); further, traces of hydrogen peroxide in the distillate reacted slowly with potassium iodide to liberate iodine. It was therefore impossible to differentiate chemically between sulphur dioxide and the other volatile iodine-reducing substances present in the distillate obtained by steam-distillation.

3. A physical separation of the volatile iodine-reducing substances, which produce the cider blank value, from sulphur dioxide was achieved by distillation under reflux, as in the alkalimetric procedures. The combination of iodimetric titration with distillation under reflux in a stream of nitrogen is made possible by the fact that the vapour pressure of iodine in potassium iodide solution is greatly reduced by increasing the concentration of potassium iodide. In the proposed method the small amount of iodine volatilised by passing nitrogen through 0·01 N iodine in 5 per cent. potassium iodide solution is completely absorbed in a second tube containing 5 per cent. potassium iodide. This second tube also acts as a second absorber for sulphur dioxide, for which purpose a trace of iodine is initially added to the contents. Since it is essential that no iodine should escape from the second tube, a third absorption tube is included, containing 5 per cent. potassium iodide solution and a drop of starch indicator. This tube normally remains colourless during several determinations.

4. During the distillation, a small amount of the sulphur dioxide re-combines (presumably in the vapour phase) with acetaldehyde, which also distils over. In order to determine this bound sulphur dioxide, the combined contents of tubes 1 and 2 are made weakly alkaline (pH 8·3) to allow the acetaldehyde bisulphite to dissociate. After 30 seconds the solution is acidified and the residual iodine titrated with 0·01 N sodium thiosulphate. Control experiments showed that acetaldehyde itself is not oxidised by iodine under these conditions.

5. In the alkalimetric methods described by Paul[10] and by Kielhöfer and Aumann[9] the sulphur dioxide is carried over in a stream of air. The good recoveries quoted by them are surprising (as recognised by Kielhöfer and Aumann), since most other workers have found it necessary to carry out the distillation in the presence of carbon dioxide or nitrogen. The necessity of avoiding oxidation in the method described below was established by comparing recoveries of sulphur dioxide from 10 ml of a solution containing 245 p.p.m. When the sulphur dioxide was carried over in a stream of nitrogen, the recovery was 245·5 p.p.m.; 223·5 p.p.m. were recovered when a stream of air was used.

APPARATUS—

The apparatus (see Fig. 1) is based on the Monier Williams principle of distillation under reflux in a stream of a carrier gas. It is similar to the Lieb - Zacherl apparatus used by Paul, except that the latter has only one absorption tube. The flask (round-bottomed, 250 ml, with side inlet), double-walled condenser (B24 joints) and tubes (200 mm × 25 mm) are standard pieces of glassware, obtainable from Messrs. Quickfit and Quartz, Ltd. The absorption heads are made from B24 cone-joints with the internal delivery tubes drawn out to a jet. The separate absorption units are joined together by small lengths of polyvinyl chloride tubing. Blood urea distillation tubes, obtainable from Messrs. Quickfit and Quartz, Ltd., can be used but are more difficult to rinse.

The flask is heated by a micro-bunsen burner, with a shield of asbestos with a hole (1¼ inches in diameter) being used to prevent charring of the sample. The absorption tubes are shielded from the heat of the bunsen burner by a vertical sheet of asbestos.

The nitrogen supply is taken from a cylinder of oxygen-free nitrogen, reduced to a pressure of about 15 cm of water.

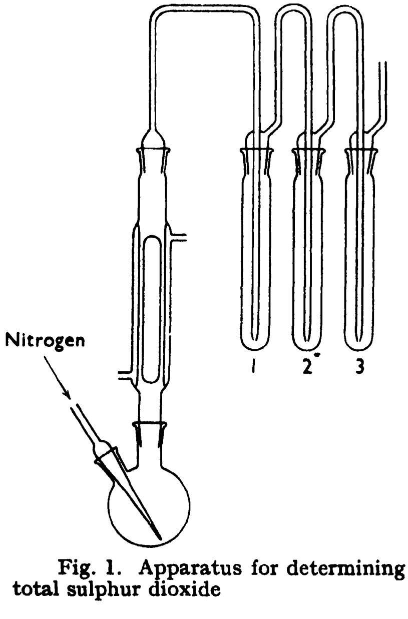

Fig. 1. Apparatus for determining total sulphur dioxide

REAGENTS—

All materials should be of analytical-reagent grade.

Iodine, 0·01 N *in 5 per cent. w/v potassium iodide solution*—This need not be freshly prepared and can be kept as a stock solution.

Potassium iodide, 5 per cent. w/v—Freshly prepare free from iodine.

Sodium thiosulphate, 0·1 N—Standardise accurately against potassium iodate. Dilute to 0·01 N as required for use.

Sodium phosphate, 0·2 M—A solution containing 72 g of disodium hydrogen orthophosphate, $Na_2HPO_4.12H_2O$, per litre.

Orthophosphoric acid, 25 per cent. v/v—A solution containing 250 ml of 85 per cent. orthophosphoric acid per litre.

Sulphuric acid, N.

Starch indicator—Prepare a fresh 1 per cent. w/v solution of soluble starch.

PROCEDURE—

By pipette transfer 5-ml portions of 5 per cent. potassium iodide solution to tubes 2 and 3, and add one drop of starch indicator to the contents of tube 3; transfer 10 ml of 0·01 N iodine to tube 1.

By pipette place the sample (10 or 20 ml, containing up to 3 mg of sulphur dioxide) in the 250-ml flask, and add 5 ml of 25 per cent. phosphoric acid. Attach the flask to the condenser. Adjust the flow of nitrogen to a moderate rate (2 to 3 bubbles per second). Heat the flask for 15 minutes with a small flame, and gently boil the contents.

Remove the flame, but keep the nitrogen flowing. Disconnect tube 2, and rinse the delivery tube. Similarly disconnect and rinse tube 1.

Pour and rinse the contents of tube 2 into tube 1, and add 5 ml of 0·2 M sodium phosphate solution. After 30 seconds add 2 ml of N sulphuric acid, and titrate the excess of iodine with 0·01 N sodium thiosulphate solution, adding 5 drops of starch indicator near the end-point. During the titration it is convenient to stir the solution in the tube with a magnetic stirrer having a small polythene-coated iron rod (1 cm long). Subtract the volume of titre from the volume of sodium thiosulphate equivalent to 10 ml of 0·01 N iodine *plus* 5 ml of 5 per cent. potassium iodide found by direct titration. One millilitre of 0·01 N sodium thiosulphate is equivalent to 0·32 mg of sulphur dioxide.

NOTES—

1. A blank titration, carried out by distilling 20 ml of distilled water instead of the sample, should agree with the direct titration of 10 ml of 0·01 N iodine to within ±0·02 ml of sodium thiosulphate solution. This shows that any iodine volatilised from tube 1 is completely retained in tube 2. This is confirmed by the fact that the contents of tube 3 normally remain colourless, and therefore do not need to be replaced during several determinations.

2. Duplicate determinations usually agree within ±0·02 ml of 0·01 N sodium thiosulphate, equivalent to 0·6 p.p.m. of sulphur dioxide in a 10-ml sample.

3. For samples containing more than 3 mg of sulphur dioxide, the volume of 0·01 N iodine in tube 1 can be suitably increased. Similarly, for extremely small amounts of sulphur dioxide the volume of iodine can be reduced, but 5 per cent. potassium iodide solution should then be added to keep a total volume of 10 ml.

4. Samples in which most of the sulphur dioxide is in the free state (*i.e.*, not combined as bisulphite compounds) rapidly evolve their sulphur dioxide in the early stages of boiling, and traces of sulphur dioxide may fail to be absorbed in tube 1. With such samples it is advisable to prolong the flow of nitrogen before starting to heat the solution, so that part of the free sulphur dioxide is carried over in the cold and sufficient iodine is volatilised into tube 2 to retain any sulphur dioxide escaping past tube 1. Alternatively, 2 or 3 drops of 0·01 N iodine may be placed in tube 2 from the pipette before the remainder is placed in tube 1.

TESTS OF RECOVERY OF SULPHUR DIOXIDE

The accuracy of the method was established by investigating (*a*) recovery of free sulphur dioxide from sodium bisulphite solution, (*b*) recovery of bound sulphur dioxide from acetaldehyde sodium bisulphite added to cider and (*c*) the magnitude of the cider blank value due to volatile iodine-reducing substances other than sulphur dioxide.

A solution of sodium bisulphite, calculated to contain approximately 250 p.p.m. of sulphur dioxide, was assayed by the distillation method and by direct iodine titration. In the latter procedure, 10 ml of solution was added by pipette, with stirring, to 10 ml of 0·01 N iodine and 5 ml of 5 per cent. potassium iodide solution; the excess of iodine was then titrated as in the distillation method. Blank determinations on 10 ml of distilled water were made, both by distillation and by direct titration. The results expressed as millilitres of 0·01 N sodium thiosulphate solution were—

	Distillation	Direct titration
10 ml of distilled water	9·76, 9·74	9·75, 9·74
10 ml of sodium bisulphite	2·08, 2·10	2·09, 2·10

It can be seen that free sulphur dioxide is completely recovered by the distillation procedure, but, in order to ensure this, it is important to have the initial trace of iodine in tube 2 (see Note 4 above). The close agreement between the blank values obtained by distillation and by direct titration supports the observation in Note 1 above.

Recovery of bound sulphur dioxide from acetaldehyde bisulphite added to cider, was tested on three ciders that were shown to be completely free from sulphur dioxide (by distillation from acid solution and determination of sulphur dioxide by the colorimetric pararosaniline - formaldehyde method). Results are shown in Table I.

TABLE I

RECOVERY OF BOUND SULPHUR DIOXIDE FROM ACETALDEHYDE BISULPHITE ADDED TO CIDER

Recovery expressed as a percentage of the results obtained by direct titration

	Cider A			Cider B			Cider C*		
Procedure (see text)	Thiosulphate titre, ml	Sulphur dioxide found, mg	Recovery, %	Thiosulphate titre, ml	Sulphur dioxide found, mg	Recovery, %	Thiosulphate titre, ml	Sulphur dioxide found, mg	Recovery, %
I	7·42	2·375	100·0	7·43	2·378	100·0	8·14	2·605	100·0
	7·44	2·380		7·42	2·375		8·12	2·599	
II	7·43	2·378	100·0	7·40	2·370	99·7	8·11	2·595	99·8
	7·42	2·375	99·9	7·41	2·372	99·8	8·12	2·599	99·9
III	7·34	2·350	98·8	7·30	2·336	98·3	8·02	2·565	98·7
	7·35	2·352	98·9	7·28	2·330	98·0	8·00	2·560	98·4
IV	0·01	0·003	—	0·02	0·006	—	0·02	0·006	—
	0·01	0·003	—	0·02	0·006	—	0·02	0·006	—
V	0·01	0·003	—	0·00	0·000	—	0·00	0·000	—
	0·01	0·003	—	0·00	0·000	—	0·00	0·000	—
VI	0·01	0·003	—	0·01	0·003	—	0·01	0·003	—
	0·01	0·003	—	0·02	0·006	—	0·01	0·003	—

* The acetaldehyde bisulphite solution used with cider C was different from that used with ciders A and B.

Portions (10 ml) of acetaldehyde bisulphite solution, prepared from sodium metabisulphite and redistilled acetaldehyde, were added by pipette to 10 ml of 0·01 N iodine and 5 ml of 5 per cent. potassium iodide solution and titrated as described in the distillation method (Procedure I). Further 10-ml portions were distilled in the presence of 10 ml of cider and 5 ml of 25 per cent. phosphoric acid, and the sulphur dioxide was determined (*a*) by the full procedure described above and (*b*) by omitting the treatment with 0·2 M sodium phosphate. The former (Procedure II) gave complete recovery of sulphur dioxide, but when sodium phosphate was omitted (Procedure III) recovery was slightly low, owing to recombination of sulphur dioxide with acetaldehyde. Blank distillations (Procedure V) were also made on 20-ml portions of the ciders to ensure that none of the sulphur dioxide recorded in the previous distillations was due to other iodine-reducing substances.

Although these three ciders showed zero blank values, it cannot be assumed that all ciders would behave similarly, and some means of measuring the blank value of a cider containing sulphur dioxide is needed. Paul[10] added a trace of hydrogen peroxide to the contents of the flask in order to oxidise the sulphur dioxide before distillation. In Procedure IV, therefore, 10 ml of acetaldehyde bisulphite solution, 10 ml of cider and 5 ml of 25 per cent. phosphoric acid (*i.e.*, as in Procedure II) were treated with 10 drops of 1 per cent. w/v hydrogen peroxide before distillation; the sulphur dioxide was completely oxidised, and the blank values were negligible. In Procedure VI, 20 ml of cider, without added acetaldehyde bisulphite, were oxidised with hydrogen peroxide before distillation; the blank values were again negligible. Thus, although hydrogen peroxide was found to increase the amount of steam-voltaile iodine-reducing compounds (see Note 2 under "General Principles," p. 305), these substances were effectively retained by the reflux condenser in the apparatus described.

For ordinary purposes and with normal ciders, there seems to be no need to carry out routine blank determinations. The above treatment with hydrogen peroxide would only be needed if other evidence suggested the presence of volatile iodine-reducing substances that might interfere with the determination of sulphur dioxide.

REPRODUCIBILITY—

The method gives highly reproducible results; seven replicate determinations on 20-ml portions of a cider, containing 130 p.p.m. of sulphur dioxide, gave results between 8·06 and 8·08 ml of 0·01 N sodium thiosulphate, with a coefficient of variation of only 0·10 per cent.

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The Spectrophotometric Determination of Caffeine in Coffee and Tea Products, with Special Reference to Coffee and Chicory Mixtures

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A spectrophotometric method that can be applied to coffee and chicory mixtures is described for determining caffeine. The sample is first extracted with boiling water and then filtered on a magnesium oxide - filter-aid column. The caffeine content of the sample is calculated from the difference between the optical densities at 273 mμ of the clarified extract before and after extraction with chloroform. High results were obtained when caffeine was determined in mixtures of coffee and chicory owing to the presence in roasted chicory of 5-hydroxymethylfurfural, which was removed from the clarified water extracts by reduction with aluminium amalgam. This method gave results that agreed closely with those obtained by a semi-micro method, and there was a considerable saving in time.

THE use of a spectrophotometer provides a simple and rapid method for determining caffeine in tea and coffee and their products because of the characteristic absorption peak of caffeine at 272–3 mμ. Most methods used for determining caffeine involve the preparation of a suitably clarified water extract from which the caffeine is extracted with chloroform. After purification, the chloroform extract is weighed or its caffeine content is obtained from a Kjeldahl determination of nitrogen. This determination can, however, be avoided by spectrophotometric measurement of the chloroform extract, but, as chloroform is an unsuitable solvent for spectrophotometry, we found it advisable to confine all spectrophotometric measurements to aqueous solutions. First an examination was made of suitable methods for preparing clarified aqueous extracts of the samples. Ishler, Finucane and Borker's method[1] for removing interfering substances, by using heavy magnesium oxide and zinc ferrocyanide, was found by us to remove chlorogenic acid, but did not remove trigonelline, which has an absorption peak at 266 mμ, close to the 273-mμ peak of caffeine. We finally based our clarification technique on that used by Bower, Anderson and Titus,[2] and used a column of heavy magnesium oxide and diatomaceous filter-aid. Portions of the aqueous extracts were used for spectrophotometric measurements made on suitably diluted solutions both before and after extraction of caffeine with chloroform. The caffeine contents of the solutions were then obtained from the changes in optical density after extraction with chloroform. We found this more convenient and less liable to errors than removal of the chloroform by distillation and solution of the residue in water. Trigonelline, being insoluble in chloroform, remained in the aqueous solutions and by this procedure did not cause interference. To check these results caffeine was also determined in portions of the same clarified aqueous extracts by extraction with chloroform and then by micro-Kjeldahl determination of nitrogen in the chloroform extracts by the original method.[2]

Satisfactory agreement was obtained between the results by the two methods on samples of coffee and tea, but the spectrophotometric method gave results that were too high and variable with samples containing both coffee and chicory. Lee Kum-Tatt[3] has also reported the presence of substances interfering with the spectrophotometric determination of caffeine in coffee mixtures containing chicory, which made it necessary for him to determine the nitrogen contents of the chloroform extracts of such samples.

We have found that a sample of pure chicory gave an apparent caffeine content of 1·5 per cent. determined by the spectrophotometric method, owing to the presence in chicory of substances soluble in chloroform and having an absorption maximum at 282 mμ. We have now established that the interference is due mainly to 5-hydroxymethylfurfural, and to a lesser extent to furfural. Both these compounds have been identified among the aromatic principles of roasted chicory by Reichstein and Beitter.[4] We found that two-dimensional paper partition chromatography of a chloroform extract of roasted chicory, first with n-butanol - acetic acid - water (63 : 10 : 27) and then with 2 per cent. acetic acid, gave one spot having a similar R_F value to those of 5-hydroxymethylfurfural and which contributed almost entirely to the absorption at 282 mμ. It appeared as a dark spot in ultraviolet light of wavelength 253·7 mμ and as a yellow spot in daylight after exposure to ammonia vapour, and gave other colour reactions characteristic of 5-hydroxymethylfurfural. Thus the apparently high caffeine contents of samples of coffee and chicory mixtures were accounted for, being due to the presence of 5-hydroxymethylfurfural in the clarified extracts and to its removal by extraction with chloroform affecting the optical density at 273 mμ.

A search was then made for a simple method of removing the interference due to 5-hydroxymethylfurfural from the clarified extracts. Complete elimination was not readily obtained by steam-distillation or ether extraction, but almost complete elimination of the absorption maximum of 5-hydroxymethylfurfural at 282 mμ was obtained by reduction with aluminium amalgam, as reported by Mizuguchi *et al.*[5] It was found that treatment of pure aqueous solutions with aluminium amalgam for 30 minutes at 100° C (on a boiling-water bath) almost completely eliminated the absorption due to 5-hydroxymethylfurfural, and only slightly reduced the absorption due to caffeine, *viz.*, by about 5 per cent. The spectrophotometric method used for determining caffeine was therefore modified for products containing chicory, the extracts being reduced with aluminium amalgam before extraction with chloroform. As a result, the apparent "caffeine" content of chicory was reduced from as much as 1·5 per cent. to as little as 0·07 per cent. In the same way, the interference was removed from extracts of coffee and chicory mixtures. Aluminium amalgam reduction also made small but significant differences to the optical densities of extracts of pure coffee, confirming

TABLE I

CAFFEINE CONTENT OF SAMPLES OF COFFEE, CHICORY AND TEA

		Caffeine content found by—		
			proposed spectrophotometric method	
Sample	Sample number	micro-Kjeldahl method, %	without reduction, %	with reduction, %
Roasted coffee	1	1·25	1·43	1·30
	2	1·99	—	1·94
	3	2·01	—	2·07
	4	2·19	—	2·06
	5	1·94	—	2·03
	6	1·92	—	2·03
	7	1·56	—	1·50
Instant coffee	1	3·26	—	3·51
	2	3·62	—	3·79
"Caffeine-free" instant coffee		0·60	0·52	0·48
Roasted chicory		0·00	1·54	0·07
Coffee and chicory mixture (1 + 1)		0·68	—	0·74
Dried extracts of coffee and chicory with carbohydrate	1	0·35	—	0·37
	2	0·43	1·36	0·47
	3	1·02	—	0·98
	4	1·10	—	1·03
Liquid coffee and chicory essences	1	0·26	0·64	0·25
	2	0·20	0·55	0·25
	3	0·35	0·38	0·35
	4	0·30	—	0·30
Ceylon tea BOPF		2·75	2·88	—
Assam tea PF		3·62	3·64	—
Ceylon tea BOP		2·75	2·80	—
Darjeeling tea		4·17	4·16	—
Instant tea		7·92	8·05	—
Instant tea with carbohydrate		3·08	3·10	—

the presence of small amounts of furfural compounds in coffee (which was also confirmed by paper chromatography); the results of the caffeine determinations then agreed more closely with those obtained by the micro-Kjeldahl method. When this method was used for determining caffeine in samples of tea, which contains no 5-hydroxymethylfurfural, the reduction stage was omitted as being unnecessary.

In making the spectrophotometric measurements, corrections were made for background absorption by use of the "base line" procedure of Morton and Stubbs,[6] deducting the mean of the readings obtained at 250 and 296 mμ (*viz.*, at wavelengths on the ascending and descending portions of the extinction curve at equal intervals on either side of the maximum at 273 mμ) from the reading at 273 mμ. The results of the determinations of caffeine in roasted coffee, and in products containing coffee and chicory, obtained by the proposed spectrophotometric method were in close agreement with those obtained by micro-Kjeldahl determinations. Similarly, with the omission of the aluminium amalgam reduction stage, there was close agreement in the caffeine contents of samples of tea determined by the two methods (see Table I).

METHOD

PREPARATION OF THE EXTRACTS—

Weigh accurately in a beaker a suitable amount of the sample, according to its expected caffeine content (*e.g.*, 1 g of coffee, 0·5 g of tea or 0·25 g of dried coffee extract). Add 3 g of heavy magnesium oxide and 15 ml of boiling water, and stand the beaker on the boiling-water bath for at least 10 minutes, stirring at intervals with a glass rod.

Prepare a packed column in a Soxhlet filter tube (180 mm × 22 mm) with a sintered-glass plate (porosity 2) with a suspension in water of 5 g of a mixture of equal weights of heavy magnesium oxide and a diatomaceous filter-aid (*e.g.*, Dicalite or Celite) by suction under reduced pressure; ensure that the column is not sucked dry in the process.

Transfer the contents of the beaker to the column with boiling water, and filter under reduced pressure. Continue washing the column with boiling water until at least 150 ml of filtrate have been collected (ensure that the column is not sucked dry). Transfer the extract with water to a 500-ml conical flask, add 5 ml of 10 per cent. v/v sulphuric acid, and boil for 5 minutes or until the volume of liquid has been reduced to about 150 ml. Cool, transfer the extract to a 200-ml calibrated flask with water, and dilute to the mark.

REDUCTION WITH ALUMINIUM AMALGAM—

Prepare the aluminium amalgam as described below. Cut sheet aluminium (0·02 inches thick) into pieces about 1 cm square, and clean the surface by heating in 10 per cent. sodium hydroxide solution on a water bath until there is an evolution of hydrogen. Wash with distilled water and then with 95 per cent. ethanol. Cover with 2 per cent. mercuric chloride solution for 3 minutes to allow a film of amalgam to form on the surface. Decant off the solution, wash the aluminium amalgam with distilled water and then with 95 per cent. ethanol, and store in ethanol until required for use.

By pipette, transfer 20 ml of the prepared extract of the sample to a beaker, add one piece of aluminium amalgam, and stand the beaker on a boiling-water bath for 30 minutes. Cool the beaker, and filter the contents through a Whatman No. 41 filter-paper into a 50-ml calibrated flask, and dilute to the mark (solution *a*).

PREPARATION OF THE SOLUTIONS FOR SPECTROPHOTOMETRIC MEASUREMENT—

By pipette transfer 10 ml of solution *a* to a 50-ml calibrated flask, and dilute to the mark (solution *b*). Measure 20 ml of solution *a* into a separating funnel, extract 5 times, each time with 15 ml of chloroform, and discard the chloroform extracts. Transfer the contents of the separating funnel to a beaker with water, boil to expel any residual chloroform, and reduce the volume to less than 50 ml. Cool, transfer the contents of the beaker to a 50-ml calibrated flask with water, and dilute to the mark (solution *c*).

SPECTROPHOTOMETRIC READINGS AND CALCULATIONS—

Measure the optical densities (E) of solutions *b* and *c* in 1-cm silica cells against distilled water with a spectrophotometer, such as a Unicam SP500, at 250, 273 and 296 mμ*; calculate the caffeine content, C, of the sample from the equation—

$$C = \frac{(2E_b - E_c)\,1250}{410 \times w}$$

where w = weight of sample taken and E_b and E_c are the values of—

$$E_{273} - \frac{(E_{250} + E_{296})}{2}$$

for solutions *b* and *c*, respectively. The value of this expression for a 0·001 per cent. solution of caffeine is 0·410.

RESULTS

The results shown in Table I have been obtained on samples of coffee, coffee and chicory mixtures, dry and liquid extracts of coffee and chicory, tea and dried extracts of tea. Caffeine was determined by Bower, Anderson and Titus's (micro-Kjeldahl) method,[2] and by the proposed spectrophotometric method.

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* The wavelength of maximum absorption and the optical density of a 0·001 per cent. solution of pure caffeine should be checked for each instrument used for determining caffeine by this method.

2,2'-Thiodiethanethiol as a Colorimetric Reagent for the Determination of Nickel

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A quantitative spectrophotometric method has been developed for determining nickel with 2,2'-thiodiethanethiol. In ammoniacal medium the reagent forms a red complex with nickel, insoluble in water and soluble in chloroform, which is suitable for determining the metal in the concentration range 2 to 25 p.p.m. with an error of 1 to 3 per cent. Copper, cobalt and palladium interfere with the determination; however, these ions can be removed by standard techniques before the analysis. No other ions were found to interfere.

THIS paper describes an investigation of a new colorimetric reagent, 2,2'-thiodiethanethiol, for determining nickel. In procedures involving use of dimethylglyoxime,[1] α-furildioxime[2] or cyclohexane-1,2-dione dioxime,[3] iron interferes and has to be complexed or removed before the colorimetric determination. Quinoxaline-2,3-dithiol[4] appears to be the only reagent reported for nickel with which this interference is not encountered. However, the limited stability of this reagent in solution is disadvantageous.

The preparation and the synthesis of 2,2'-thiodiethanethiol (TDT) have been described by Mathias[5] and Harley-Mason.[6] The latter used ammoniacal nickel salt solutions and thiourea for determining mustard gas in air; a coloured complex formed between nickel and TDT, which was generated *in situ*.

TDT is not specific for nickel alone, but the reagent permits the determination of this metal in the presence of many other ions. The complex is extracted from the aqueous phase with a water immiscible solvent, such as chloroform.

METHOD

REAGENTS—

2,2'-Thiodiethanethiol, $HSCH_2CH_2SCH_2CH_2SH$, is a colourless liquid, boiling at 138° C (13 mm), having a specific gravity of 1·191 at 20° C and a refractive index of 1·5958 at 20° C. It is immiscible with water, but soluble in halogenated organic solvents. The compound is not available commercially, but can be synthesised readily by the procedure described by Mathias.[5]

2,2'-Thiodiethanethiol solution, 0·2 M—Prepared by diluting 12·7 ml of TDT to 500 ml with chloroform.

Standard nickel solutions—A stock solution of nickel sulphate (approximately 0·05 M) was prepared by dissolving 7·2709 g of analytical-reagent grade nickel sulphate heptahydrate in water, and diluting to 500 ml with water. One millilitre of this solution was found to contain (by gravimetric analysis with dimethylglyoxime) 3·23 mg of nickel.

Ammonium hydroxide solution, 25 per cent. w/w, aqueous—Analytical-reagent grade.

Chloroform—Analytical-reagent grade.

APPARATUS—

Spectrophotometric measurements were made in 1-cm glass cells with a Zeiss model PMQ II spectrophotometer at a slit width of 0·01 cm. The measurements were made at 25° C.

PROCEDURE—

Dilute a 20·0-ml portion of the standard nickel solution to 1000 ml in a calibrated flask; transfer 2·0-, 5·0-, 10·0-, 20·0- and 25·0-ml portions to 50- to 100-ml separating funnels. Add ammonium hydroxide solution dropwise until the pH is 10 to 11, and then add 5 to 10 ml of the 0·2 M TDT solution. Shake for a few minutes, then run off the lower organic phase. Extract the aqueous phase three times, with a different portion of the reagent each time, and then wash the residual aqueous phase with 5- to 10-ml portions of chloroform, and separate. Collect the organic phases in 50-ml calibrated flasks, and dilute to the mark with chloroform. Measure the optical densities of these pink to red solutions at 520 mμ against pure chloroform. Plot a graph relating the optical density to the amount of nickel present; the graph should be linear and pass through the origin.

Samples containing approximately 0·1 to 1 mg of nickel should be analysed in the same way as described for the preparation of the calibration graph, the concentration of nickel in the sample being read from the calibration curve.

RESULTS AND DISCUSSION

The nickel complex of 2,2′-thiodiethanethiol in chloroform is red, whereas the solution of the reagent itself is colourless. The optical-density spectrum for the coloured complex shows a maximum at 520 mμ.

The formation of the complex is dependent on the pH of the aqueous solution. Fig. 1 indicates that a pH above 10 is needed for quantitative formation of the complex between nickel and TDT.

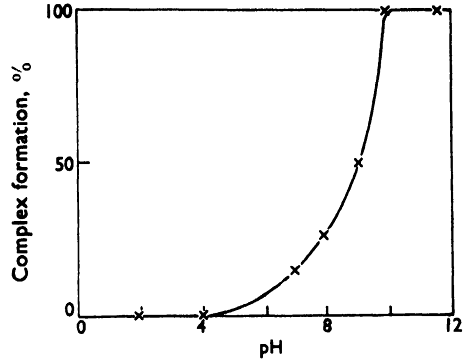

Fig. 1. Complex formation as a function of pH

The optical density of the solution of the complex in chloroform is stable for a long period of time. Solutions measured immediately after their preparation and again after 1 or 2 weeks did not show any appreciable change in optical density (see Table I).

TABLE I

COLOUR STABILITY AS A FUNCTION OF TIME

Solution A contained 3·8 p.p.m. of Ni^{2+}; solution B contained 12·9 p.p.m. of Ni^{2+}

Time after preparation of solution, weeks	Optical density of Solution A	Optical density of Solution B
0	0·075	0·234
1	0·072	not determined
2	not determined	0·231

Samples of the reagent as well as samples of solutions of the reagent in chloroform were kept at room temperature in glass-stoppered bottles for 12 months with no change in concentration or any deterioration.

Job's method of continuous variations[7] indicated that every atom of nickel in the complex is bound to two molecules of TDT and that a single complex species is formed in solution.

EFFECT OF OTHER IONS—

Cobalt and palladium interfere in the determination of nickel with TDT by forming coloured complexes equally soluble in chloroform. Copper also interferes, possibly by oxidising the TDT to its disulphide and thus repressing the reaction with the nickel. However, many of the common ions have no effect on the determination (see Table II).

TABLE II

EFFECT OF VARIOUS IONS ON THE DETERMINATION OF NICKEL

Ion	Ratio of foreign ion to nickel by weight	Error in nickel determination, %
Na^+	5	−0·3
K^+	5	−0·3
Ca^{2+}	0·3	−0·3
Mg^{2+}	5	−0·3
Pb^{2+}	3·5	+1·0
Zn^{2+}	2	0
Sn^{2+}	4	+1·5
Mn^{2+}	25	0
Sb^{3+}	4	0
Fe^{3+}	5	−0·3
Cr^{3+}	9	0
Al^{3+}	1	−0·3
Cl^-	25	−0·3
NO_3^-	1000	+0·6
CH_3COO^-	6	0
SO_4^{2-}	9	0
PO_4^{3-}	30	0

The interference of cobalt, palladium and copper can be overcome by using one of the known methods for their removal. Thus the complexes of cobalt and palladium with ammonium thiocyanate[3] are extracted with ethyl acetate and pentanol, respectively, and copper is removed by precipitation with thioglycollic acid.[8] The residual solutions can then be used for determining nickel. Table III shows the results obtained for the determination of nickel, in the presence of interfering ions, by these modifications of the procedure.

TABLE III

DETERMINATION OF NICKEL IN THE PRESENCE OF INTERFERING IONS

Interfering ion	Ratio of foreign ion to nickel by weight	Error in nickel determination, %
Co^{2+}	3	+0·6
Pd^{2+}	4	0
Cu^{2+}	40	+1·7

ACCURACY OF DETERMINATION—

The versatility and applicability of this analytical procedure were investigated by determining nickel in standard samples of the National Bureau of Standards (U.S.A.). After the sample had been dissolved in the usual way, the nickel steel, No. 33d, and the chromium - molybdenum steel, No. 133, were analysed without prior removal of any ion. The copper - nickel - zinc alloy, No. 157, and the manganese bronze, No. 62c, were analysed after precipitation of the copper with thioglycollic acid. Tables IV to VII summarise the results of these analyses.

TABLE IV

DETERMINATION OF NICKEL IN NICKEL STEEL STANDARD SAMPLE 33d*

In each test the portion taken for the measurement of optical density was 2 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·2608	0·142	3·7	+2·8
	0·142	3·7	+2·8
0·2678	0·140	3·6	0
0·3023	0·160	3·6	0
	0·161	3·6	0
		Average ..	+1·1

* Nickel steel 33d contains: 95 per cent. of Fe, 0·5 per cent. of Mn, 0·25 per cent. of Mo, 0·25 per cent. of Si, 0·17 per cent. of C, 0·14 per cent. of Cr, 0·12 per cent. of Cu and 3·6 per cent. of Ni.

TABLE V

DETERMINATION OF NICKEL IN CHROMIUM - MOLYBDENUM STEEL 133*

In each test the portion taken for the measurement of optical density was 10 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·4787	0·093	0·27	− 6·9
	0·103	0·29	0
0·4945	0·110	0·30	+ 3·5
	0·113	0·31	+ 6·9
0·4994	0·115	0·32	+10·0
	0·116	0·32	+10·0
		Average ..	+ 3·9

* Chromium - molybdenum steel 133 contains: 83·8 per cent. of Fe, 13·6 per cent. of Cr, 0·8 per cent. of Mn, 0·6 per cent. of Mo, 0·4 per cent. of Si, 0·4 per cent. of S, 0·1 per cent. of C and 0·29 per cent. of Ni.

TABLE VI

DETERMINATION OF NICKEL IN COPPER - NICKEL - ZINC ALLOY STANDARD SAMPLE 157*

In each test the portion taken for the measurement of optical density was 1 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·1539	0·200	17·9	0
	0·203	18·3	+2·2
0·1549	0·210	18·7	+4·5
	0·214	18·8	+5·0
0·1584	0·208	18·0	+0·6
		Average ..	+2·5

* Copper - nickel - zinc alloy 157 contains: 72·1 per cent. of Cu, 9·7 per cent. of Zn, 0·14 per cent. of Co, 0·05 per cent. of Fe and 17·9 per cent. of Ni.

TABLE VII

DETERMINATION OF NICKEL IN MANGANESE - BRONZE STANDARD SAMPLE 62c*

Weight of sample, g	Portion taken, ml	Optical density at 520 mμ	Nickel found, %	Error, %
0·4264	5 from 25	0·090	0·29	0
	10 from 25	0·200	0·28	−3·5
0·4938	10 from 25	0·210	0·29	0
	5 from 25	0·106	0·29	0
0·4983	5 from 25	0·107	0·30	+3·5
0·5029	5 from 25	0·115	0·31	+6·9
0·5120	10 from 25	0·220	0·29	0
			Average ..	+1·0

* Manganese bronze 62c contains: 59·2 per cent. of Cu, 37·2 per cent. of Zn, 1·2 per cent. of Al, 0·7 per cent. of Fe, 0·7 per cent. of Mn, 0·4 per cent. of Sn, 0·2 per cent. of Pb, 0·07 per cent. of Si and 0·29 per cent. of Ni.

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SHORT PAPERS

Colorimetric Determination of Low Concentrations of 2,2-Dichlorovinyl Dimethyl Phosphate in the Atmosphere

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2,2-Dichlorovinyl dimethyl phosphate (DDVP) is an anticholinesterase insecticide of relatively high volatility (vapour pressure = 0·1 mm of mercury at 20° C) and moderately low mammalian toxicity.[1] In addition to its use in conventional spraying or aerosol formulations, or in baits, the compound may also be effectively applied purely as a fumigant by incorporation in a suitable base, such as polyvinyl chloride, from which it is continuously released as a vapour into the atmosphere.[2] To investigate the use of DDVP as a fumigant against mushroom pests, a method was required for determining atmospheric concentrations of 0·1 μg per litre or less i cropping houses and other enclosed spaces. The method reported by Geiger and Fürer,[3] in which the test solution is treated with resorcinol and sodium carbonate, fails to distinguish between DDVP and any free dichloroacetaldehyde present, and apparently determines both together. Although the method is sensitive it also suffers from the further disadvantage of producing an unstable coloured product and a somewhat inconsistent and high reagent blank value. A method is reported here for determining low concentrations of both unhydrolysed DDVP and free dichloroacetaldehyde present in the same aqueous solution. A stable coloured product is formed, and the reagent blank value is low.

The method is based on that already proposed by Hodgson and Casida[4] for dichloroacetaldehyde, in which use is made of its reaction with 2,4-dinitrophenylhydrazine, the product forming a blue solution on treatment with an excess of alkali and a mutual solvent. The reaction conditions have been modified to increase the over-all sensitivity of the method, and, by introducing a controlled hydrolysis stage, it has been found possible to determine microgram amounts of both dichloroacetaldehyde and DDVP present in the same solution. Hodgson and Casida[4] state that hydrolysis of the vinyl - phosphate bond of DDVP is complete in 0·2 N sodium hydroxide in 15 minutes at 27° C. However, determinations of the dichloroacetaldehyde produced from known amounts of pure DDVP, hydrolysed under these conditions, showed recoveries of only 50 per cent. or less, losses evidently being due to secondary alkaline decomposition of the dichloroacetaldehyde. Geiger and Fürer[3] found, by measuring the amount of alkali consumed, that hydrolysis of DDVP is quantitatively complete if carried out at 0° to 1° C for 20 minutes in N/6 sodium hydroxide. Measurements by the proposed colorimetric procedure of the hydrolysis of pure DDVP under closely similar conditions (15 minutes in 0·2 N sodium hydroxide at 0° C) confirmed that the recovery—even with microgram amounts—was substantially complete. Tests with known amounts of dichloroacetaldehyde ranging from 3·4 to 27·5 μg submitted to the latter hydrolysis conditions indicated a constant recovery of at least 90 per cent.

As shown by Hodgson and Casida,[4] DDVP does not readily hydrolyse and react with 2,4-dinitrophenylhydrazine, under mild treatment, so that if conditions are correctly chosen the free dichloroacetaldehyde can be determined in the presence of the ester. A further determination on some of the same solution after quantitative hydrolysis gives a measure of the total aldehyde present. By difference, the amount of unchanged DDVP originally present can be calculated.

To test the usefulness of the method a series of mixtures of DDVP and dichloroacetaldehyde was prepared and analysed. Dichloroacetaldehyde was prepared from dichloroacetal by the method proposed by De Bièvre and co-workers.[5] Although the aldehyde polymerised readily when stored at room temperature, and slowly at −20° C, de-polymerisation could be effected by distillation and the freshly distilled product (b.p. 89·9° C) was immediately dissolved in water. Pure samples of DDVP were kindly provided by the Shell Chemical Company Ltd. and Ciba Laboratories Ltd.

Results obtained on a series of mixtures, each equivalent to about 60 μg of DDVP, are shown in Table I.

Experiments to check recoveries of low concentrations in the atmosphere have been carried out by evaporating 0·01- and 0·1-ml portions of dilute aqueous solutions of DDVP in 1- and 10-litre

TABLE I

AMOUNTS OF DDVP AND DICHLOROACETALDEHYDE FOUND IN PREPARED MIXTURES

Mixture	DDVP—		Dichloroacetaldehyde—	
	taken, µg	found, µg	taken, µg	found, µg
1	60·0	58·2	0	0·4
2	56·0	52·3	3·0	3·4
3	45·0	44·0	7·5	8·0
4	30·0	29·3	15·0	15·3
5	15·0	13·9	22·5	22·9
6	6·0	5·2	27·0	27·8
7	0	0	30·0	30·5

glass vessels. Metered volumes of air were drawn through the vessels, which were fitted with taps, into three bubblers containing distilled water. The mean recovery obtained, in 5 experiments, with simulated mean air concentrations ranging from 0·16 to 4·6 µg per litre, was 93 per cent. At the lowest concentration 70 per cent. of the total was recovered as DDVP, the mean for the series being 83 per cent. Most of the recovered material was always found in the first absorber. Under the least favourable conditions of sampling used (350 litres at a rate of 0·5 to 1 litre per minute) the proportions found in the first, second and third absorbers were 0·84, 0·14 and 0·02, respectively.

To obtain a convenient optical-density reading in the test, a volume of at least 0·25 cu. metre would be required for an air sample containing 0·1 µg of DDVP per litre. To take sample volumes in excess of 1 cu. metre (which can be taken overnight) would in general require inconveniently long times, so that for practical purposes the lowest measurable concentration can be regarded as about 0·02 µg per litre.

METHOD

SAMPLING—

Aspirate a sufficient volume of the air through two or three bubblers in series, each containing 10 ml of distilled water, by means of a suitable pump. The rate should not exceed 1 litre per minute, and the volume of the air may be measured with a gas meter or other device.

DETERMINATION OF TOTAL DICHLOROACETALDEHYDE—

Adjust the sample volume to 10 ml. Place a 5-ml portion of each sample solution in a 10-ml graduated tube, and immerse this in crushed ice until the temperature is reduced to 0° C. Add 1·0 ml of 1·2 N sodium hydroxide (the temperature of which has previously been adjusted to 0° C), mix, and maintain at 0° C for exactly 15 minutes. Add 0·6 ml of 0·1 per cent. 2,4-dinitrophenylhydrazine solution in 4 N hydrochloric acid, mix, and place in a water bath at 37° C for 60 minutes. Add 0·6 ml of 4 N sodium hydroxide solution, shake, add ethanol to give a volume of approximately 10 ml, and mix thoroughly. Cool to 20° C, and adjust the volume to exactly 10 ml with ethanol. Measure the optical density at 580 mµ against a reagent blank solution. Determine the amount of dichloroacetaldehyde present by reference to a calibration graph obtained from known amounts of dichloroacetaldehyde submitted to the same procedure, including the hydrolysis stage.

DETERMINATION OF FREE DICHLOROACETALDEHYDE—

To the remaining 5 ml of sample in each bubbler add 1·0 ml of water and 0·6 ml of 0·1 per cent. 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid, and maintain at 37° C for 60 minutes. Complete the procedure as before, and determine the dichloroacetaldehyde present by reference to a calibration graph obtained with known amounts of dichloroacetaldehyde.

Calculate the amount of combined dichloroacetaldehyde present by difference, and express this as DDVP by applying the theoretical conversion factor (1·956).

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A Method for the Rapid Determination of Alcohol in Body Fluids

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VARIOUS methods of determining alcohol in body fluids have been described in recent years. Kent-Jones and Taylor[1] reported the results of an investigation into the merits of two methods—the micro Cavett and that of Kozelka and Hine. The micro Cavett method was found to be the more accurate, but it suffered from serious inconsistencies in reproducibility; Kozelka and Hine's method, although less accurate, gave good reproducibility but was arduous and time-consuming.

Nickolls[2] reported a modification of the micro Cavett method that appears to give results as accurate as those obtained by the unmodified method and even better reproducibility than that given by Kozelka and Hine's method. The simplicity of the procedure recommended its use for routine work in the laboratory.

In this laboratory work is in progress to assess the acclimatisation of cattle to tropical environments, and one of the important variables being considered is the change in total body water content at various environmental temperatures. Budtz-Olsen, Cleeve and Oelrichs[3] investigated the total body water of sheep, and recommended the use of alcohol, on the dilution principle, as offering the simplest means of determining this parameter. In preliminary investigations Nickolls's method has been simplified (*a*) by reducing the time for the determination and (*b*) by assessing the reduction of dichromate photometrically.

METHOD

APPARATUS—

Jars—Kilner jars (1-lb size) fitted with two rubber gaskets.

Glass triangles with 1-inch legs.

Petri dishes—5 cm in diameter.

Incubator—Temperature controlled at approximately 45° C.

REAGENT—

Potassium dichromate solution—Dissolve 4·9 g of potassium dichromate in 1 litre of 50 per cent. v/v sulphuric acid.

PROCEDURE—

By pipette put 10 ml of potassium dichromate solution into a Kilner jar, place a glass triangle in the jar, and on top of the triangle place a Petri dish. From a pipette place 2 ml of blood or other body fluid containing the alcohol in the Petri dish, and then add 2 ml of water directly on top of the blood to ensure a good mixture. Place the two rubber washers and the glass top of the jar in position, and screw on the metal cap tightly. (It is of the utmost importance that the jars be completely airtight.) Place the jar in an oven controlled at 45° C for 2 hours, then remove it, and set it aside to cool. When it has cooled, open the jar and remove the Petri dish. Wash the legs of the glass triangle with distilled water from a wash bottle, and then remove the triangle from the jar. Transfer the contents of the jar, with several washings, to a 100-ml calibrated flask, and make up to the mark with distilled water.

Measure the optical density of the solution in a 1-cm cuvette with a spectrophotometer at 450 mμ; use distilled water in the reference cuvette. Construct a calibration graph by measuring the reducing effect of known weights of alcohol on the dichromate solution. A straight line is produced for amounts of alcohol between 0 and 3·5 mg. A calibration graph must be constructed with each new batch of alcohol or dichromate solution.

The results of tests to determine the accuracy of the method in which three lots of blood containing different amounts of alcohol were analysed are shown in Table I. A sample of blood without added alcohol was included with each series, and in no instance was there any reduction of dichromate.

TABLE I

RESULTS

Alcohol content of blood, mg per 2 ml	No. of determinations	Mean alcohol recovered, mg	Standard deviation
0·600	20	0·613	0·030
1·708	20	1·694	0·034
2·996	20	3·006	0·016

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A Rapid Method for determining Sulphate in Water Extracts of Soils

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THIS paper describes modifications of Butters and Chenery's method[1] (for determining total sulphur in soils and plants) that increase its sensitivity and make it suitable for determining sulphate in water extracts of soils.

The main modification involves the use of an extremely dilute seed suspension of barium sulphate added to the test solution. This was done since it was found that, in the original method when the portion taken contained less than 10 µg of sulphate-sulphur, no absorption occurred. The use of the seed suspension overcame this and gave good recovery of as little as 2 µg of sulphate-sulphur. Further, the sensitivity of the method was also increased, and as little as 2 p.p.m. of soil sulphate-sulphur could be determined with a fair degree of accuracy.

It was also found that the use of barium chloride crystals of less than 1-mm sieve size without removal of particles less than 52 mesh, as described in the original method, gave more consistent results. The final main modification involves the incorporation of the gum acacia reagent with the acetic acid reagent. This mixed reagent was stable indefinitely, whereas the aqueous gum acacia solution described in the original method tended to become turbid after a few days.

Since several other minor modifications were also made, the method finally adopted for determining sulphate in water extracts of soils is described in full below.

METHOD

REAGENTS—

Nitric acid, 25 per cent. v/v—Prepare from analytical-reagent grade nitric acid.

Acetic - phosphoric acid—Mix 900 ml of analytical-reagent grade acetic acid with 300 ml of analytical-reagent grade orthophosphoric acid.

Gum acacia - acetic acid solution—Dissolve 5 g of gum acacia in 500 ml of hot water. Filter the hot solution through a Whatman No. 42 filter-paper, cool the filtrate, and then dilute to 1 litre with analytical-reagent grade acetic acid.

Barium sulphate seed suspension—Dissolve 18 g of analytical-reagent grade barium chloride in 44 ml of hot water, and add 0·5 ml of the concentrated standard sulphur solution (see below). Bring this to the boil, and then cool quickly. Then add 4 ml of the gum acacia - acetic acid solution. Prepare this seed suspension freshly each day before use.

Barium chloride—Grind analytical-reagent grade barium chloride to pass a 1-mm sieve.

Concentrated standard sulphur solution, 2 mg per ml—Dissolve 1·088 g of oven-dried analytical-reagent grade potassium sulphate in water, and dilute the solution to 100 ml.

Working standard sulphur solution, 10 µg per ml—Each day, dilute 5 ml of the concentrated standard to 1 litre with water.

Purified animal charcoal—Boil 20 g of Norit NK charcoal with about 200 ml of concentrated hydrochloric acid for about 10 minutes, filter under suction, and wash until free of chloride. Dry in an oven at about 100° C.

PROCEDURE—

Place 10 g of soil (air-dried and ground to pass a 2-mm sieve) and 20 ml of water in a 6-inch × 1-inch boiling tube. Stopper with a rubber bung, and shake in a mechanical reciprocating shaker for 15 minutes. Add 0·04 g of purified animal charcoal, and shake for another 15 minutes. Filter through a Whatman No. 42 filter-paper; use dry apparatus and filter-paper. Place a suitable portion of the filtrate (not more than 15 ml and containing not more than 120 μg of sulphate-sulphur) in a 6-inch × ¾-inch test-tube calibrated at 25 ml, add 2·5 ml of 25 per cent. nitric acid and 2 ml of acetic - phosphoric acid, and dilute to about 22 ml. Insert the bung, shake the tube, and then add, successively, 0·5 ml of the barium sulphate seed suspension (which must be shaken just before use) and 1 g of barium chloride crystals. Insert the bung, and invert the tube 3 times. After 10 minutes invert 10 times, and after another 5 minutes invert 5 times. After another 5 minutes add 1 ml of the gum acacia - acetic acid solution. Dilute to volume, invert 3 times, and set aside for 1½ hours. Then invert the tube 10 times, pour the contents into a 4-cm cell, and measure the optical density with a Hilger Biochem absorptiometer; use the dark blue filter (optimum transmission at approximately 440 mμ).

PREPARATION OF STANDARDS—

Place 0, 1, 3, 5, 8, 10 and 12 ml of the working standard sulphur solution in separate 25-ml calibrated test-tubes, and continue as described above. Prepare standards each time a batch of solutions is analysed, and also run a reagent blank test.

The addition of charcoal during extraction usually produces a colourless extract. When this does not occur, treat a portion of the solution with all the reagents except barium chloride and the barium sulphate seed suspension. Subtract the optical-density value obtained with this from that obtained with another portion given the normal full reagent treatment. This will allow for any absorption owing to colour in the extract.

Preliminary tests showed that the charcoal does not absorb any sulphate from the extract.

RESULTS AND DISCUSSION

The method was tested by determining the recovery of known amounts of sulphate added to water extracts of thirty different soils (varying widely in pH, texture and organic matter content, and including six soils containing free carbonates). Recovery ranged from 96·4 to 103·1 per cent. and averaged 101·2 per cent.

The modified method described here is about five times more sensitive than the original, thus it is particularly suitable for determining sulphate in soils where sulphate-sulphur content is low compared with that of total sulphur.[2,3] However, there is no reason why it cannot be applied to determining total sulphur in soils and plant material after the digestion procedures described by Butters and Chenery in their original method.

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Determination of Small Amounts of Long-chain Aliphatic Tertiary Amines in Aqueous Solution

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ASHBROOK'S[1] method for determining tri-iso-octylamine in aqueous raffinates has been modified by Lloyd and Carr[2] to make it applicable for some other tertiary and secondary amines. In an attempt to evolve a more sensitive method, we observed that ferric thiocyanate is superior to the cobalt thiocyanate complex in the determination of some tertiary amines. The method consists essentially in extracting the highly coloured ferric thiocyanate - amine complex in an organic solvent for spectrophotometric determination. Cyclohexane was chosen from several solvents as the extractant. The method was found to be suitable for the two tertiary amines—tri-iso-octylamine and trinonylamine (TNA), but not so sensitive for the secondary amines Amberlite LA-1 and LA-2; no other types of amines were available.

EXPERIMENTAL

DEVELOPMENT AND EXTRACTION OF THE COMPLEX—

With known amounts of either of the tertiary amines in the aqueous solution, the extraction of the ferric thiocyanate - amine complex, as indicated by the optical density of the organic extract measured against cyclohexane at 490 mμ, was found to depend on the concentrations of ferric and thiocyanate ions in the aqueous phase, as well as on its pH. When the aqueous phase was 0·1 M in ammonium ferric sulphate and M in ammonium thiocyanate and adjusted to pH 1·5, maximum extraction was obtained at a ratio of organic to aqueous phase of not less than 1 to 5. Under these conditions the optical density of the organic extract varied linearly with concentration of the amine up to 0·8 mg in 10·0 ml.

EFFECT OF OTHER IONS ON COLOUR FORMATION—

During the processing of aqueous leach liquors[3] for recovery of uranium by solvent extraction, the raffinates contain, in addition to dissolved and entrained amine, several ionic impurities, such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, HSO_4^- and $H_2PO_4^-$. Interference from some of these ions in the final determination of the amine was studied, and it was found that, although HSO_4^-, $H_2PO_4^-$ and Fe^{3+} could be tolerated when present up to 80, 5 and 5 g per litre, respectively, Cu^{2+} gave lower values when present to the extent of as little as 0·5 g per litre. The presence of such impurities as dissolved silica and flocculating agents,[4] such as glue or Separan, was found to cause a further decrease in the optical density of the extract. It was found, however, that by first extracting the amine with cyclohexane and then developing the colour in the organic layer by allowing it to come into contact with an aqueous solution containing ferric thiocyanate, interference from several ions likely to be present in aqueous solution could be avoided. Based on these observations, the procedure described below is recommended for determining the two tertiary amines in aqueous samples.

METHOD

PROCEDURE—

By pipette, place a suitable portion of the sample solution (up to 50 ml), containing 0·05 to 0·5 mg of the amine, in a 100-ml separating funnel. Add 10·0 ml of cyclohexane, and shake vigorously for 2 minutes. Allow the phases to separate, and discard the aqueous phase. Add 1 ml of M ammonium ferric sulphate, 5 ml of 2 M ammonium thiocyanate, 0·5 ml of M sulphuric acid and about 5 ml of distilled water. Shake for 1 minute, and then allow the phases to separate. Discard the aqueous phase, and spin the organic phase in a centrifuge. Measure the optical density of the extract in a 1-cm cell at 490 mμ against cyclohexane. Read the amount of amine from a calibration graph prepared by adding known portions of a solution of the amine in methanol to 25 ml of distilled water and carrying out the same procedure as described for the samples.

RESULTS

Tests were carried out to verify the method in which known amounts of the amines were added to a synthetic raffinate solution, *i.e.*, an aqueous solution prepared by mixing different salts to give a final composition corresponding to a typical raffinate sample from solvent-extraction tests.

The results of triplicate analyses of two of the raffinate samples obtained in solvent-extraction tests on a laboratory mixer - settler unit are shown in Table I.

TABLE I

DETERMINATION OF TERTIARY AMINES IN SIMULATED RAFFINATES

The approximate composition per litre of each raffinate was 50 g of SO_4^{2-}, 5 g of Cl^-, 5 g of PO_4^{3-}, 1 g of Cu^{2+}, 2 g of Fe^{2+}, 10 g of Fe^{3+}, 3 g of Al^{3+}, 0·5 g of Ni^{2+}, 5 g of Mn^{2+} and 1·5 g of SiO_2

Sample	Amine found, mg per litre	Amine expected,* mg per litre
I (tri-iso-octylamine)	20·0, 19·7, 19·8	20·0
II (trinonylamine)	7·8, 8·0, 7·6	7·8

* Determined by the volumetric[5] and a modified cobalt thiocyanate method.

The results show that the two amines could be determined by the proposed method. The relative sensitivity of the method, compared with other methods, is shown in Table II.

TABLE II

COMPARISON OF THE METHODS WITH REFERENCE TO SENSITIVITY

Amine	Amount of amine, mg	Organic phase (10 ml)	Optical density (1-cm cell) Lloyd and Carr's method	Modified cobalt thiocyanate method	Proposed method
XE204	1	Pentanol and kerosene	0·10	—	—
Tri-iso-octylamine	1	Carbon tetrachloride	—	0·133	—
Trinonylamine . .	1	Carbon tetrachloride	—	0·133	—
Tri-iso-octylamine	1	Cyclohexane	—	—	1·67
Trinonylamine . .	1	Cyclohexane	—	—	1·70

It is clear that the advantage of the method lies in its greater sensitivity than that of Lloyd and Carr and the modified cobalt thiocyanate method.

We are grateful to Shri H. N. Sethna, Head, Chemical Engineering Division, and Shri S. Fareeduddin, Officer-in-Charge, Ore Extraction Section, Atomic Energy Establishment, Trombay, for facilities to carry out the work and permission to publish the results.

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The Determination of Sodium in Halo-phosphate Phosphors by Atomic-absorption Spectroscopy

BY J. PERKINS

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THE phosphors currently used in the production of fluorescent lamps are of the calcium halo-phosphate type, prepared by firing together calcium hydrogen phosphate, calcium carbonate and a lesser amount of calcium fluoride. In order to study the effect of contamination by sodium on the performance of these phosphors, it was necessary to develop a method for determining the sodium content.

Direct flame photometry is unsatisfactory owing to the presence of strong band spectra from both calcium and phosphate ions in the region of the sodium lines at 5890 and 5896 Å. Even with an instrument having fairly high wavelength selectivity, *e.g.*, the Uvispek H700 spectrophotometer, the background level is unacceptably high. Hibbs and McDonald[1] have employed a preliminary chemical separation and then flame photometry for determining sodium in mixed alkaline-earth carbonates. The application of this method removes completely the calcium and phosphate, but owing, probably, to the gelatinous nature of the calcium phosphate precipitate, co-precipitation of sodium occurs and extremely poor recoveries of known additions are obtained. In view of the advantages in selectivity reported in the literature,[2] it was decided to investigate the possibility of using atomic-absorption spectroscopy for this determination.

EXPERIMENTAL

The apparatus used was a Uvispek H700 spectrophotometer with an H868 flame-photometer attachment. The flame chimney was modified so that light could be passed through the flame and thence into the spectrophotometer. The source of resonance radiation used was a Mazda 60-watt 250-volt sodium discharge lamp, which was under-run at 125 volts, from a Variac autotransformer, in the interest of greater stability and reduced line width.

The solutions under test were sprayed into the oxy-hydrogen flame in the normal way, and the absorption was measured at 589 mμ, the photometer being set to zero absorption (100 per cent. transmission) while distilled water was being sprayed.

CALIBRATION—

A stock solution containing 100 μg of sodium per ml was prepared by dissolving 0·051 g of sodium chloride in 200 ml of de-ionised water, and from this a series of standards containing from 10 to 50 μg of sodium per ml, in dilute hydrochloric acid (1 + 19), was prepared. These were used to calibrate the instrument, and, since the emission from the flame at these concentrations was negligible compared with the radiation from the sodium lamp, a linear response was obtained over the range 0 to 50 μg per ml; the results were—

Concentration of sodium, μg per ml	10	20	30	40	50
Absorption at 589 mμ	0·07	0·14	0·21	0·28	0·35

INTERFERENCE—

Standard additions of sodium were made to solutions containing (*a*) 1 per cent. w/v of calcium (as calcium chloride) in dilute hydrochloric acid (1 + 19), (*b*) 1 per cent. w/v of phosphate (as orthophosphoric acid) in dilute hydrochloric acid (1 + 19) and (*c*) 0·5 per cent. w/v of calcium *plus* 0·5 per cent. w/v of phosphate in dilute hydrochloric acid (1 + 19).

Determinations of sodium were carried out on these solutions by atomic absorption and by emission flame photometry with a Uvispek H700 spectrophotometer. The results in Table I show the marked superiority of the atomic absorption method in respect of interference.

TABLE I

EFFECT OF CALCIUM AND PHOSPHATE ON THE RECOVERY OF SODIUM

			Sodium found by—	
Interfering ions	Concentration of interfering ions, %	Sodium added, μg per ml	emission flame photometry, μg per ml	atomic-absorption spectroscopy, μg per ml
Calcium	1	0	8	<0·5
		20	28	18
		50	60	49
Phosphate	1	0	2	<0·5
		20	21	18
		50	52	48
Calcium + phosphate	0·5 + 0·5	0	4	<0·5
		20	23	19
		50	54	49

SENSITIVITY—

It was considered that the smallest real absorption that could be measured was 0·003, representing a sodium concentration of 0·5 μg per ml. On a 0·25-g sample of phosphor in 10 ml of solution this corresponds to a sodium content of 20 p.p.m. This was shown to be sufficiently sensitive for our purpose, as can be seen from subsequent results.

METHOD

PROCEDURE—

Dissolve a suitable weight, but not more than 0·25 g, of phosphor in the least possible amount of concentrated hydrochloric acid in a platinum crucible. Evaporate gently until salts just begin to crystallise. Dissolve in about 5 ml of de-ionised water, and add 0·5 ml of concentrated hydrochloric acid. Make up to 10 ml in a calibrated flask with de-ionised water, and return the solution at once to the platinum crucible to avoid pick-up of sodium from the glass. Spray the solution into the flame from a silica or platinum crucible, and measure the absorption at 589 mμ. Determine the sodium content of the sample by reference to a graph prepared as described under "Calibration." (This is best checked at one point, say 20 μg per ml, with each determination, as variations in the flame conditions or lamp temperature significantly affect the sensitivity.)

RESULTS

The sodium content of three phosphors was determined, and three further determinations were made after known amounts of sodium had been added to each. A 0·25-g sample was used for each determination; the results were—

	Sample A		Sample B		Sample C	
Sodium added, µg	Nil	50	Nil	200	Nil	200
Sodium found, µg	20, 19	69	150, 150	345	170, 160	365

To test the reproducibility of the method, replicate determinations were carried out on two samples, one of low and one of high sodium content and standard deviations were calculated; the results were—

	Sample D	Sample E
Sodium found, µg	25, 24·5, 22·5, 22·5 24·5, 23, 24, 22·5	275, 282, 278, 284 284, 280, 283, 283
Standard deviation, µg ..	1·1	3·3

CONCLUSION

The method described has proved satisfactory for determining sodium in halo-phosphate phosphors, and many samples have been successfully analysed. It would, with suitable modifications, be applicable to other materials, where for reasons of interference from other species, emission flame photometry is impracticable.

The time taken for a single determination is approximately 15 minutes.

REFERENCES

1. Hibbs, J. M., and McDonald, G. W., *Analyst*, 1960, **85**, 846.
2. David, D. J., *Ibid.*, 1960, **85**, 779.

Received *August 3rd*, 1962

Elimination of the Water Effect on Argon Ionisation Detectors fitted to Pye Chromatographs

BY J. L. MARTIN

(*Glaxo Laboratories Ltd., Greenford, Middlesex*)

To obtain quantitative results for small amounts of alcohols in aqueous solutions from a gas chromatograph fitted with an argon ionisation detector, it is necessary to prevent water passing into the detector. If this is not done the drop in sensitivity, which is proportionate to the amount of water and the temperature of the detector, reduces the peak areas of components subsequently eluted. The back-flushing technique[1] is not conveniently applied at temperatures approaching 100° C if the substance to be measured has a larger retention volume than water (even when the extremely polar diglycerol is used as stationary phase), and the device described was introduced primarily for determining 0·02 to 0·25 per cent. of 2-phenylethanol in aqueous solutions. However, the problem of determining small amounts of relatively non-volatile substances in aqueous solution is one that is frequently met in pharmaceutical analysis, and the modification has proved of general use.

It was thought desirable temporarily to divert the gas flow during the time that it was carrying water vapour, so that the detector would be by-passed. It is difficult to contrive a tap at the lower end of the column that can be manipulated during elution, owing to the particular geometry of the Pye instrument used; the column itself was therefore used as a tap control as described below.

The adaptor consists of as short a length of Pyrex-glass tubing as possible (to reduce dead space to a minimum), fitted with a B7 socket at one end (the top) and a B7 cone at the other (see Fig. 1 (*a*)). The B7 socket is grooved lengthwise internally, and a hole is also drilled in it 180° from the groove. The lower B7 cone of the chromatographic column to be used is modified so that the only exit for gas is a hole drilled in the side of the cone: the hole coincides, when column and adaptor are assembled, with the hole in the adaptor socket.

When the two holes are juxtaposed in use (Fig. 1 (*b*)), the argon, together with any water separated on the standard 4-foot column, is vented to the atmosphere, leaving the detector unaffected. When the argon gas flow has to be diverted again into the ionisation cell, the column is turned through 180° without stopping the flow, and the hole in the column cone then corresponds with one end of the groove leading to the detector (Fig. 1 (*c*)). A preliminary chromatogram must be prepared, to indicate at what time the column should be turned.

Benzyl alcohol, chlorocresol, trichloroethanol and the esters of *p*-hydroxybenzoic acid have been determined by means of the modification; it has also proved useful during the conditioning of chromatographic columns by removal of the volatile impurities from them.

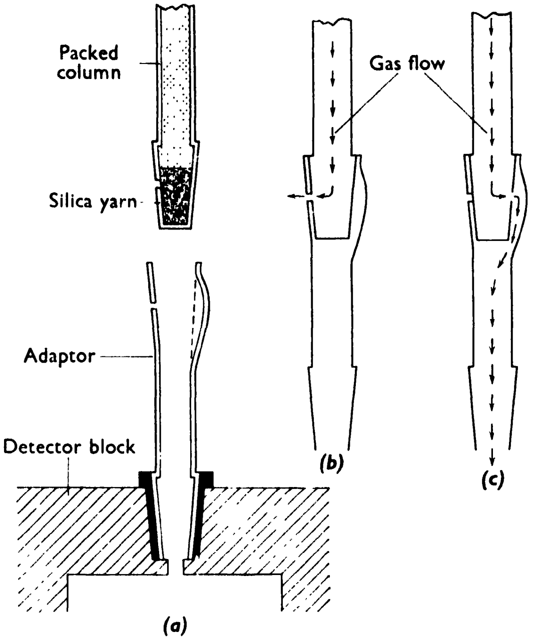

Fig. 1. Diagram of adaptor

REFERENCE

1. Swoboda, P. A. T., *Chem. & Ind.*, 1960, 1262.

Received *August* 31*st*, 1962

Book Reviews

ABSORPTION SPECTROSCOPY. By ROBERT P. BAUMANN. Pp. xiv + 611. New York and London: John Wiley & Sons Inc. 1962. Price 94s.

The most impressive single feature of this book is its wide scope. As explained in the author's preface, it is intended to be of value in three distinct types of educational courses in molecular spectroscopy. The first is designed for the specialist worker concerned with fundamental studies of molecular structure and dynamics, the second for the "applied" spectroscopist, whose major interests are qualitative and quantitative analysis, and the third is the provision of background information on molecular spectroscopy for the industrial analyst with no previous formal training in the subject. This classification is a valid one. Undergraduate instruction in the fundamentals of molecular spectroscopy is necessarily limited to the consideration of selected aspects of theory and application, if only because of time limitations and the demands of other important topics for attention. Conversely, "applied" molecular spectroscopy has often been dealt with on an essentially empirical basis, so that both the power of the underlying theory and the great value of relatively simple qualitative concepts have not always been emphasised. The demand of the general analyst for an introduction to molecular spectroscopy arises from the fact that present-day commercial spectroscopic equipment, with high standards of performance, is now sufficiently simple to operate that, in principle at least, obtaining reliable results no longer requires a long apprenticeship in spectroscopic techniques.

To a notable extent this book does cover the field of absorption spectroscopy in a manner that meets, in large measure, two of the three stated objectives. Since these aims involve the exposition of the subject both in breadth and depth they account for a certain unevenness in the treatment. Thus the problems that occur at the end of each chapter range from trivial numerical calculations, through problems in the physics of radiation and the optics of spectrometers, to examples of quantum theory and molecular dynamics. The problems provided clearly demonstrate the way in which the subject is being developed in depth, but the "general analyst" reader may have been tempted to give up before reaching Chapter 8, "Qualitative Analysis" (pp. 314 to 363). Here the treatment of qualitative analysis by electronic (visible and ultraviolet) and infrared absorption spectroscopy, although lucid, is probably too brief to be of real value and has been done better elsewhere; even so the 19 problems to this chapter provide a useful test of competence in this field and are well worth looking at. Chapter 9, "Quantitative Analysis" (pp. 364 to 433), contains useful sections on transmittance error functions in relation to minimum concentration errors, expanded scale operation, the examination of ideal and non-ideal multi-component systems (including a detailed matrix treatment for a 4-component case), and a useful series of problems. Possible errors due to stray radiation effects in electronic absorption spectra are not dealt with, however, and the treatment of this important topic in Chapter 3, "Spectrometer Design and Performance," is too brief to be of direct practical value.

The remainder of the book comprises a long section, Chapter 10, "Principles of Molecular Spectroscopy" (pp. 434 to 544), supplemented by appendixes on matrix methods, character tables, etc., and selected answers to many of the problems. In this Chapter the theoretical aspects of the subjects dealt with earlier in Chapters 5 to 7 (pp. 202 to 313) are developed in detail, with sections on group theory and molecular symmetry, electronic spectra, vibrational spectra, rotational spectra and interactions between rotational, vibrational and electronic transitions. The discussion of these topics is concise and formal, without much explicit reference to illustrative spectral data.

These remarks should be sufficient to indicate that this book covers a large proportion of the basic theory of molecular spectroscopy, and thus goes a considerable way towards satisfying the demands of potential specialist workers. The discussion of the instrumental, practical and applied aspects is also excellent, despite some deficiencies. The chapter on "Spectrometer Components" is noteworthy in providing an outline of the physical principles on which instrument design and performance are based.

It may be doubted, however, if the book is suitable as an introduction to molecular spectroscopy for the newcomer with no previous experience of the subject. The wide scope of the book and the depth to which particular topics are explored would make it heavy reading for this type of student. But for the other classes of readers at which it is aimed, it constitutes an impressive combination of theoretical exposition and practical instruction. G. H. BEAVEN

METHODS OF BIOCHEMICAL ANALYSIS. Volume X. Edited by DAVID GLICK. Pp. x + 399. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1962. Price 109s.

This volume opens with an account by C. T. Bishop (of Ottawa) on separation of carbohydrate derivatives by gas - liquid partition chromatography. The widest use of the procedures described is in analysing methanolysis products from methylated polysaccharides, but other possible applications are noted. The method is, however, limited to compounds having the necessary volatility and supports rather than replaces other techniques.

R. and Ruth E. Benesch (of Columbia University) contribute a critical account of the determination of –SH groups in proteins and conclude that all existing methods have limitations and that several methods should be used on each protein.

The measurement of sodium and potassium by glass electrodes is discussed by S. M. Friedman (of Vancouver). This is a rather new field with considerable possibilities. The selectivity of cation-specific glasses for one alkali metal cation relative to another is reproducibly dependent on the composition of the glasses and appears to depend on the Na (or Li)/Al (or B) ratio of the lattice. Commercial glass electrodes of the desired type are now appearing, and the instrumentation needed is "not particularly difficult," either for experiments *in vitro* or *in vivo*.

H. G. Pontis and Leloir (of Buenos Aires) describe the measurement of uridine diphosphate (UDP) - enzyme systems. The various enzyme systems connected with UDP compounds are outlined, and the reliability of the assay procedure "has been checked by several investigators." This is a clear and authoritative contribution.

C. S. Vestling (of Urbana, Illinois) deals with the determination of dissociation constants for two-substrate enzyme systems, such as alcohol dehydrogenase, lactic dehydrogenase and malic dehydrogenase, which are DPN^+-linked. Here the DPN^+ (or nicotine adenine dinucleotide NAD) may properly be regarded as a second substrate. Various experimental approaches are discussed (ultracentrifugation, dialysis, spectrophotometry, fluorescence) and there is a section covering the theoretical and experimental aspects of kinetic determinations of dissociation constants.

L. C. Craig and T. P. King (of the Rockefeller Institute) contribute an article on dialysis in which, in the main, seamless cellulose tubing is used. The porosity can be decreased by acetylation for definite times or it can be increased by treatment with zinc chloride solution. The use of a series of cells permits counter-current dialysis. The same authors in another chapter also discuss counter-current distribution in a masterly way with examples of many biochemical applications.

P. A. Albertson (of Uppsala) has an interesting chapter on partition methods for fractionation of cell particles and macromolecules. The methods used are polymer phase systems, such as dextran - polyethylene glycol - H_2O or dextran - methylcellulose - H_2O, in which the viscosities are not too high. The materials studied are viruses, proteins, nucleoproteins and nucleic acids. Among the examples of purifications described are those of bacteriophage T and polio virus.

The determination and microscopic localisation of cholesterol is the subject of a chapter by J. J. Kabara (of Detroit). This is an excellent and fully documented account of an important and difficult problem.

K. Yagi (of Nagoya, Japan) surveys the chemical methods for the determination of flavins. There is first a good account of the background of the subject and this is followed by details of extraction methods from animal and plant tissues. Estimations of total flavins by absorption or fluorescence spectrophotometry are then described and a further section discusses clearly the separation and determination of individual flavins.

This volume maintains the standard set in the earlier volumes. The international character of the publication is noteworthy, as is the absence this time of any contributor from this country.

R. A. Morton

The Properties and Testing of Plastics Materials. By A. E. Lever, A.I.R.I., and J. Rhys, M.Sc., A.K.C. Second Edition. Pp. x + 321. London: Temple Press Books. 1962. Price 55s.

"Plastics" is a word embracing an ever-growing variety of polymeric materials that may have little in common other than their man-made origin. After a basic polymer has been made it must often go through a complex series of operations to produce a material that can be used in the manufacture of every-day things, and the manufacturer of plastics must be prepared to test this material at all stages of manufacture for its suitability for a particular purpose, for it often happens that he is, in fact, selling physical properties to his customers. Although chemical analysis is required at some stages in the manufacture of plastics, by far the larger amount of testing carried out is physical in character.

As in other branches of chemical technology, information about the testing of plastics is widely scattered in the literature; the object of the book under review is to summarise this information. The value of the book lies in its classification of these tests so that, without much difficulty, the reader may quickly discover sources of information about any particular one. The first six chapters cover general principles and physical, thermal, optical, electrical and sundry chemical properties, a short chapter deals with identification and chemical analysis, twenty-one classes of industrial materials are then discussed in some detail, and the last two chapters deal with the testing of finished mouldings and the efficiency of plasticisers. Although the chapter on chemical analysis is short, further analytical information is to be found in other chapters. Intending readers should appreciate that the book is, in effect, a literature review covering over three thousand references appearing up to 1961 with, usually, only brief remarks about any particular test; no attempt is made to give the reader a critical evaluation of the methods discussed.

A. G. Jones

Publications Received

ANORGANISCHE ULTRAMIKROANALYSE. By I. P. ALIMARIN and M. N. PETRIKOVA. Pp. 169. Berlin: Veb Deutscher Verlag Der Wissenschaften. 1962. Price DM 27.

ABSORPTION SPECTRA IN THE ULTRAVIOLET AND VISIBLE REGION. Volume III. Edited by Dr. L. LÁNG, with the collaboration of Dr A. BARTECKI, Dr. J. SZÖKE, Dr. G. VARSÁNYI and M. VIZESY. Pp. 424. (loose leaf). Budapest: Akadémiai Kiadó, 1962. Price $16.00.

THIN FILM CHROMATOGRAPHY. By E. VERNON TRUTER, Ph.D., B.Sc., A.R.C.S., D.I.C. Pp. xii + 205. London: Cleaver-Hume Press Ltd. 1963. Price 37s. 6d.

GERMAN-ENGLISH CHEMICAL TERMINOLOGY: AN INTRODUCTION TO CHEMISTRY IN ENGLISH AND GERMAN. By ALEXANDER KING, C.B.E., D.Sc., A.R.C.S., D.I.C., and Prof. Dr. HANS FROMHERZ. Fourth Edition. Pp. xx + 588. Germany: Verlag Chemie. 1963. Price DM 48; $12.00.

ION FLOTATION. By FELIX SEBBA. Pp. viii + 154. Amsterdam, London and New York: Elsevier Publishing Company. 1962. Price Dfl. 15.00; 30s.; DM 17.

PHYSICAL AND CHEMICAL METHODS OF SEPARATION. By EUGENE W. BERG, Ph.D. Pp. xiv + 366. New York, San Francisco, Toronto and London: McGraw-Hill Book Company Inc. 1963. Price 97s.

CHEMICAL BONDING AND THE GEOMETRY OF MOLECULES. By GEORGE E. RYSCHKEWITSCH. Pp. x + 116. New York: Reinhold Publishing Co.; London: Chapman and Hall Ltd. 1963. Price 16s.

Errata

JANUARY (1963) ISSUE, p. 28, 25th line. *For* "0·1 N" *read* "0·01 N."
IBID., p. 61, 8th line. *For* "130·13 *VC*/178" *read* "130·13 *VC*/356."
MARCH (1963) ISSUE, p. 215, 13th line. *For* "$\nless$ 6 litres" *read* "$\nless$ 6 changes."

MAY, 1963 Vol. 88, No. 1046

THE ANALYST

EDITORIAL

Development of *The Analyst*

In February of this year the Council of the Society received and - except for one matter whose implementation required further consideration—approved a report from the *Analyst* Development Committee. This Committee had, over a period of just on two years, studied criticisms of *The Analyst* and suggestions for changes in its contents and format. It began by inviting members and subscribers, both through a general announcement in the *Bulletin* and by direct personal invitation to members known to hold decided views, to submit their ideas. Thereafter a round dozen were interviewed singly or in small groups, and what they had to say was discussed and recorded in a detailed summary of evidence: this record alone eventually occupied 60 pages of close-spaced typescript.

When the points made at these meetings had been marshalled into some sort of order, a document was drawn up for the Committee to consider in greater detail than had been possible while the ideas were still being put forward. By this stage the Committee had had much valuable discussion with the propounders of the ideas, and it was clear that on some matters there were as many views as critics, and most of them contradictory. But some points of agreement (though never unanimous) had appeared. The members of the Development Committee, too, put forward their own suggestions, and the two written contributions that had been received were also considered. The outcome of the Committee's investigations and deliberations was the presentation to council of 35 recommendations for positive actions that the Committee unanimously believed would enhance the reputation and status of *The Analyst.*

As might be expected, it took longer to consider the criticisms and weigh the suggestions than it had to make them, and during this time some of the less controversial matters were referred to Council, received favourable decisions and were implemented. Already the appearance of the journal has been improved by starting each paper on a new page and by using a modern white paper in place of the older style cream-tinted paper for the text. The second of these changes was made only after the Committee had considered specimen pages printed on eight different papers and had compared them with other journals; even then the final choice was not endorsed until tests by PATRA had shown that the new paper was in many respects technically superior to the old, and inferior in none. But other suggestions, such as changing the size of the page or altering the size of the type, were found to have positive disadvantages and were rejected, and suggestions that the number of pages in each issue should be increased, although they were sympathetically received, can only be implemented when the rate of inflow of papers has permanently increased.

It appeared that some misconceptions might prevail as to the range of subjects considered suitable for publication in *The Analyst,* and as to whether there were any restrictions on authorship. The Committee hoped that removal of these misconceptions would result in more papers being offered, and to this end they have revised the "Notice to Authors." The new version, printed on p. 409, has a preamble intended to convey the information that *The Analyst* caters for all types of paper on the theory and practice of analytical chemistry, provided only that they reach a satisfactory standard of quality. Reviews, fundamental work, descriptions of specialised techniques and applications of existing methods in new contexts—all are essential to a properly balanced journal. *The Analyst* is, too, an international journal—a recent survey showed no less than a third of the contributions to be from overseas—

and the Editors are, when necessary, ready and willing to assist authors whose natural tongue is not English with the presentation of their papers. Nor do authors need to be members of the Society—roughly twice as many papers are accepted from non-members as from members (of course, membership of the Society has many *other* advantages).

Some anxiety had been expressed to the Development Committee about the refereeing system for papers. Much of this proved to be due to a lack of information on the subject; the Committee concluded that not only had the system to be fair (which it was), but that it had to be seen to be fair (which was much more difficult to arrange). Previously, acceptances have been based on a single referee's report, whereas rejections have required two or more adverse reports, obtained seriatim at much expense of time. In future all papers will be sent simultaneously to two referees; if their reports concur, final decisions will be made at once by the Editorial Committee, and if the original reports differ, the Committee will itself select a third referee as arbiter and make its decision only after receiving the third report. The two primary referees will be selected from the existing comprehensive panel of over 200, plus the additions that are constantly being made; this system will be much more rapid, if slightly more mechanical, than hitherto.

The Editorial Committee is, basically, the former Publication Committee. Its functions include the supervision of all aspects of production of *The Analyst.* But the old Publication Committee's responsibility for policy has been transferred to a new Publications Policy Committee. This move is in keeping with the increasing number and scope of the Society's publications, which include, besides *The Analyst* and *Analytical Abstracts*, Monographs, collections of Standard Methods, publications by the Analytical Methods Committee and sundry volumes of Symposium and Congress Proceedings. It is indeed to this Publications Policy Committee that Council has passed, for urgent attention, the question of implementing the excepted recommendation mentioned in the first sentence of this editorial. Although the Development Committee has successfully planned its own demise, it has left a more permanent successor on which will rest the responsibility for further development of *The Analyst.*

Even now, only half of the Development Committee's recommendations have been implemented, and an account of some of the remaining decisions must await a future occasion.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ANNUAL GENERAL MEETING

THE eighty-ninth Annual General Meeting of the Society was held at 2.15 p.m. on Friday, March 8th, 1963, in the meeting room of the Royal Society, Burlington House, London, W.1. The Chair was occupied by the President, Dr. A. J. Amos, O.B.E., B.Sc., F.R.I.C. The Financial Statement for the year ending October 31st, 1962, was presented by the Honorary Treasurer and approved, and the Auditors for 1963 were appointed. The report of the Council for the year ending March, 1963 (see pp. 334–343), was presented by the Honorary Secretary and adopted.

The Scrutineers, Messrs. P. W. Shallis and K. L. Smith, reported that the following had been elected officers for the coming year—

President—D. C. Garratt, Ph.D., D.Sc., Hon. M.P.S., F.R.I.C.

Past Presidents serving on the Council—A. J. Amos, R. C. Chirnside, J. H. Hamence and K. A. Williams.

Vice-Presidents—S. G. Burgess and R. E. Stuckey.

Honorary Treasurer—D. T. Lewis.

Honorary Secretary—S. A. Price.

Honorary Assistant Secretaries—C. A. Johnson (Programmes Secretary) and D. W. Wilson.

Other Members of Council—The Scrutineers further reported that 143 valid ballot papers had been received. As the number of candidates for election as Ordinary Members of Council had been equal to the number of places to be filled, there had been no ballot for their election.

The President declared the following to have been elected Ordinary Members of Council for the ensuing two years—L. Brealey, A. G. Jones, E. Q. Laws, F. C. J. Poulton, S. G. E. Stevens and C. Whalley.

H. E. Brookes, P. F. S. Cartwright, B. S. Cooper, J. F. Herringshaw, R. M. Pearson and A. A. Smales, having been elected members of the Council in 1962, will, by the Society's Articles of Association, remain members of the Council for 1963.

C. J. House (Chairman of the North of England Section), R. A. Chalmers (Chairman of the Scottish Section), F. H. Pollard (Chairman of the Western Section), W. H. Stephenson (Chairman of the Midlands Section), D. W. Wilson (Chairman of the Microchemistry Group), W. Cule Davies (Chairman of the Physical Methods Group) and W. A. Broom (Chairman of the Biological Methods Group) will be *ex-officio* members of the Council for 1963.

The retiring President, Dr. Amos, thanked the Honorary Officers for their services to the Society during his term of office. He then formally installed Dr. Garratt, who for many years has been Chairman of the Analytical Methods Committee, as President.

After the business outlined above had been completed, the meeting was opened to visitors, and the retiring President delivered his Presidential Address (see pp. 344–351).

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 6.30 p.m. on Wednesday, May 1st, 1963, at University College, Gower Street, London, W.C.1. The Chair was taken by the President, Dr. D. C. Garratt, Hon.M.P.S., F.R.I.C.

The following paper was presented and discussed: "The Use of Magnetic Resonance Measurements in Chemistry," by Professor R. S. Nyholm, M.Sc., Ph.D., D.Sc., F.R.A.C.I., F.R.I.C., F.R.S.

MIDLANDS SECTION

The Eighth Annual General Meeting of the Section was held at 7 p.m. on Thursday, March 28th, 1963, at the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by Mr. W. T. Elwell, F.R.I.C. The following appointments were made for the ensuing year:—*Chairman*—Mr. W. H. Stephenson. *Vice-Chairman*—Mr. W. T. Elwell. *Hon. Secretary*—Mr. M. L. Richardson, John & E. Sturge Ltd., Lifford Chemical Works, Lifford Lane, Kings Norton, Birmingham 30. *Hon. Treasurer*—Mr. F. C. J. Poulton. *Hon. Assistant Secretary*—Mr. R. Adkins. *Members of Committee*—Prof. R. Belcher, Dr. R. G. H. B. Boddy, Mr. H. E. Brookes, Mr. W. M. Dowson, Mr. G. Ingram, Mr. N. Nix, Mr. D. M. Peake, Dr. H. C. Smith (*ex-officio* for 1 year) and Mr. C. Whalley. Miss M. E. Tunnicliffe and Mr. J. Blenkin were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section when the following paper was presented and discussed: "Reactions in Non-aqueous Solutions" by Dr. L. D. Pettit.

Annual Report of the Council: March, 1963

IT has become customary in recent Annual Reports of Council to record and comment on the increasing activities of the Society. During the present year these activities have at least been maintained.

For the second time in recent years the Society's Annual General Meeting—the eighty-eighth—was held outside London. At the invitation of the North of England Section, both the Annual General Meeting and the Bernard Dyer Memorial Lecture were held in Manchester, and Dr. D. W. Hill gave an address entitled "Research and the National Economy." Again, as in Birmingham two years previously, the lecture was well attended, including a substantial proportion of members from outside the North of England Section; the lecture was followed in the evening by the Society's Annual Dinner, again well attended by members from all parts of the country.

Although the report of the Midlands Section, given separately, refers to the Feigl Anniversary Symposium held in Birmingham in April, 1962, it is appropriate to mention it here. The Symposium was held in the Chemistry Department of the University of Birmingham and organised on behalf of the Society by the Midlands Section. Speakers and delegates from 28 countries—to a total of 400—attended to hear lectures, to join the discussions and to pay tribute to Professor Fritz Feigl. The Microchemistry Group, through their Chairman, Mr. C. Whalley, also paid tribute, from the microchemists of the United Kingdom, with the presentation of a magnificent glass vase. This Symposium is yet another in the line of successful Symposia organised at Birmingham by the Midlands Section, and all involved deserve cordial thanks for their work.

The Sections and Groups have again been active and meetings have been as frequent and widespread as in previous years. The Scottish Section organised a meeting, jointly with the North of England Section and at the invitation of the Chemistry Department of Queen's University Belfast, on a range of physico-chemical methods of analysis. In September the Microchemistry Group held a joint meeting with the Dublin and District Section of the Royal Institute of Chemistry in Trinity Hall, Dublin, the title being "Modern Trends in Small Scale Inorganic Analysis." The year was noteworthy in that the Physical Methods Group set up an Atomic Absorption Spectroscopy Discussion Panel with Mr. W. T. Elwell as Chairman and Mr. D. Moore as Honorary Secretary; the Panel held its inaugural meeting on December 12th, 1962, at which a paper entitled "Aspects of Atomic Absorption Analysis" by D. J. David, M.Sc., was read. The Council wishes success to the Physical Methods Group in this venture.

The annual conference of Honorary Secretaries was held somewhat earlier than usual, in January. In addition to the usual meeting, an informal meeting was held under the Chairmanship of Mr. C. A. Johnson, Programmes Secretary, in an attempt to obtain closer liaison with respect to the dates of meetings of the Sections and Groups and with respect to their subject matter. A similar meeting was again held in January, 1963, and further progress was made in this direction.

The Analyst Development Committee, which took the views of a considerable number of people during the course of its investigations, completed its report to Council in January, 1963. The report, which was considered by Council at a special meeting, contains numerous recommendations relating to the constitutions and functions of editorial and policy committees for *The Analyst*, the refereeing system, the contents and format of *The Analyst* and the Proceedings, advice to authors and instructions to referees.

During the year Dr. Evers retired from the Editorship of *Analytical Abstracts*. In considering the future of this publication, Council recorded their considerable debt to Dr. Evers for the part he had played in gaining for *Analytical Abstracts* the high esteem in which the publication was now held in the field of analytical chemistry. It is fully intended that *Analytical Abstracts* will in future be given the support necessary to maintain and to increase its high reputation.

The year 1962 has been busy for the President, Dr. Amos. He spent a week at the Feigl Anniversary Symposium, where, at the invitation of the Midlands Section, he took the Chair at the opening and closing sessions, and he represented the Society at the annual dinners of other Societies and at the Ramsay Dinner, at which he replied to the toast to the guests. In addition he took an active part in the organisation of the 1st International Congress of Food Science and Technology, serving as a member of the Executive Committee and as Chairman of the Publicity Committee.

The Council records with particular pleasure the award of the C.B. to the Honorary Treasurer of the Society, Dr. D. T. Lewis. Council also records with pleasure the award of the C.B.E. to Professor A. C. Frazer and Professor H. B. Nisbet, and the award of the O.B.E. to Dr. A. M. Smith.

The Society now has 2097 members, an increase of 44 over the membership of a year ago.

Long membership—The congratulations and good wishes of the Council are extended to Dr. L. E. Campbell, Major F. K. Donovan, Dr. E. B. Hughes, Dr. D. W. Kent-Jones, Mr. A. W. Starey and Mr. R. W. Sutton, O.B.E., who have completed 40 years of membership.

Deaths—The Council regrets to have to record the deaths of the following members—

A. Alcock
J. J. V. Backes
H. W. Christian
M. Corner
C. W. Cornwall
F. R. Dodd
C. L. Hinton
H. V. Horton
R. E. Jones
J. King
F. C. B. Marshall
W. J. S. Pringle
E. Russell
H. B. Salt

Society meetings—Six meetings of the Society were held during the year; the papers read and discussed were—

April, 1962, in London:

"Square-wave Polarography with Special Reference to the Analysis of Zirconium and Hafnium," by D. F. Wood, B.Sc., A.R.I.C., and R. T. Clark.

"The Determination of Gold by Extractive Titration," by A. W. Titley, B.Sc., A.R.I.C.

"An Automatic Coulometric-titration Assembly," by P. G. W. Scott, B.Sc., A.R.I.C., and T. A. Strivens, B.Sc.

May, 1962, in London, on the Determination of Sterols:

"Determination of Cholesterol for Clinical Purposes," by G. S. Boyd, Ph.D., A.H.-W.C., A.R.I.C.

"Determination of Cholesterol and its 7-Dehydro Derivatives," by J. Glover, M.Sc., Ph.D., A.R.I.C.

"Determination of Vitamin-D Secosterols," by E. Kodicek, M.D., Ph.D.

"Gas Chromatographic Examination of Sterols," by C. J. W. Brooks, Ph.D., A.R.C.S.

"The Determination of Animal Fat in Vegetable Fats by Gas Chromatographic Analysis," by K. R. Beerthuis, Dr. Chem.

"The Determination of Plant Sterols," by Professor T. W. Goodwin, D.Sc., F.R.I.C.

"Determination of Sterols of Wool Wax and Related Materials," by E. V. Truter, Ph.D., A.R.C.S., D.I.C.

October, 1962, in London, on Recent Developments in Polarography:

"Pulse Polarography," by H. M. Davis, B.Sc., A.Inst.P., A.R.I.C.

"Differential Cathode-ray Polarography," by H. I. Shalgosky, B.Sc., A.R.I.C.

"Analytical Aspects of Radio-frequency Polarography," by Dr. H. W. Nürnberg.

November, 1962, in London, on Fluoride, Teeth and the Analyst:

"The Determination of Fluorine in Inorganic Materials by Pyrohydrolysis," by H. J. Cluley, M.Sc., Ph.D., F.R.I.C.

"Fluoridation of Public Water Supplies," by J. Longwell, D.Sc., F.R.I.C., F.R.S.H.

"The Rôle of Analysis in Investigating the Mode of Action of Fluoride in Tooth Decay," by G. N. Jenkins, M.Sc., Ph.D.

December, 1962, in London, on Applications of X-ray Fluorescence:

"The Applications of X-ray Fluorescence Spectrometry in the Steel Industry," by D. F. Sermin, A.Met.

"The Determination of Lead in Air Filters, Vanadium - Nickel Ratios in Oil Ashes, and Strontium in Tap Water by the X-ray Fluorescence Spectrometer," by R. G. Stone, B.Sc., A.R.I.C.

"X-ray Fluorescence in Archaeology at the Museum Laboratory," by E. T. Hall, M.A., D.Phil.

February, 1963, in London, on Particle-size Analysis: Some Methods Used in the Sub-Sieve Range:

"The Size Analysis of Insoluble Drugs," by M. J. Thornton, B.Sc., A.R.I.C.

"Particle-size Analysis in the Formulation of Pesticides," by C. G. L. Furmidge, B.Sc., Ph.D., A.R.I.C.

"Particle Sizing in Aerosol Systems," by D. A. Blyth, B.Sc., J. M. Creasey and N. W. Wootten, B.Sc., A.Inst.P.

SECTIONS AND GROUPS

The present membership of the Sections and Groups, as will be seen from the reports that follow, is—

North of England Section	431
Scottish Section	117
Western Section	123
Midlands Section	372
Microchemistry Group	805
Physical Methods Group	904
Atomic Absorption Spectroscopy Discussion Panel	91
Biological Methods Group	331

NORTH OF ENGLAND SECTION—Membership of the Section totals 431. Because the main concentration is centred round Merseyside and Manchester, Liverpool and Manchester have again been the main centres for meetings. We record with sorrow the death of Mr. A. Alcock, Honorary Secretary during 1955.

In June, 1962, after a very successful period of office, Mr. Brian Hulme relinquished his post as Honorary Secretary and Treasurer, a post he had held since June, 1958. Mr. G. F. Longman was elected by the Committee to act as Honorary Secretary until the next Annual General Meeting.

The papers presented and discussed were—

Liverpool, January, 1962, Annual General Meeting:

"The Work of the Laboratory of the Government Chemist," by D. T. Lewis, Ph.D., D.Sc., M.R.S.H., F.R.I.C.

Manchester, April, 1962, jointly with the Biological Methods Group:

Discussion on "The Assessment of Psychostimulants," introduced by M. W. Parkes, B.Sc., Ph.D.

Llandudno, May, 1962, Summer Meeting:

"Some Experiences in Forensic Science," by G. B. Manning, B.Sc., M.B., Ch.B., F.R.I.C.

Belfast, June, 1962, jointly with the Scottish Section:

"Investigations on the Determination of Noble Metals by Oscillographic Polarography," by I. Beattie and R. J. Magee, M.Sc., Ph.D., F.R.I.C., F.I.C.I.

"Analytical Applications of the Flame Emission Spectra of Lead and Titanium," by C. L. Chakrabarti, M.Sc., A.R.I.C., R. J. Magee, M.Sc., Ph.D., F.R.I.C., F.I.C.I., and Professor C. L. Wilson, Ph.D., D.Sc., F.R.I.C., F.I.C.I.

"An Ultramicrospectrophotometric Method for the Determination of Complex Cyanides," by F. Haba and Professor C. L. Wilson, Ph.D., D.Sc., F.R.I.C., F.I.C.I.

"Differential Cathode-ray Polorography," by H. M. Davis, B.Sc., A.Inst.P., A.R.I.C.

"Instrumental Methods of Continuous Analysis," by G. Jessop, M.Sc., Ph.D.

"Applications of Vapour-phase Infra-red Spectroscopy to the Functional Group Analysis of Propoxy and Butoxy Compounds by Modified Zeisel Reactions," by D. M. W. Anderson, B.Sc., Ph.D.

"Applications of Modern Techniques in Spectroscopy," by R. A. C. Isbell, A.Inst.P.

"The Activation Analysis of High-purity Beryllium Using Penetrating Radiations," by C. A. Baker.

"Some Analytical Applications of Mass Spectrometry," by A. Quayle, M.Sc., A.R.I.C.

"A Modular Gas Chromatograph System for the Analysis of Exit Streams from Reactors and for the Application Work Required for Process Analysers," by C. W. Munday, B.Sc., A.R.I.C., and G. R. Primavesi, B.A.

"The Methylene Insertion Reaction for the Identification of Hydrocarbons by Gas Chromatography," by E. S. Lane, B.Sc., Ph.D., F.R.I.C.

Discussion on "Quantitative Gas - Liquid Chromatography in the Routine and Research Laboratories," introduced by A. F. Williams, B.Sc., F.R.I.C.

Lathom, October, 1962:

"The Analytical Laboratory in the Glass Industry," by F. Hartley, F.S.G.T., F.R.I.C.

Middlesbrough, November, 1962, jointly with the Tees-side Section of the Royal Institute of Chemistry:

"Solvent Extraction of Inorganic Compounds, Some Recent Developments," by Professor H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., L.R.A.M.

Manchester, December, 1962, jointly with the Physical Methods Group:

"Nuclear Magnetic Resonance," by Professor E. R. Andrew, M.A., Ph.D.

SCOTTISH SECTION—Membership of the Section stands at 117 against last year's total of 123.

During 1962, seven meetings have been held, five in Glasgow, one each in Edinburgh and Belfast. The Belfast meeting, a two-day Symposium, was shared with the North of England Section. Two other meetings were held jointly with local Sections of other Societies: the November meeting with the Society of Chemical Industry, and the December meeting, as usual, conjointly by all four Chartered Bodies.

Audience numbers are greater, it seems, when the subject is of a more general nature rather than from a specific part of chemistry. The Annual General Meeting was not well attended, and it has been decided that no lecture will be given in future after the luncheon and business meeting.

The Section Committee records with pleasure the presence of the President of the Society at the Ramsay Dinner, when he ably replied to "The Guests."

The papers presented and discussed were—

Glasgow, January, 1962, Annual General Meeting:

"Applications of Analysis to Research Problems in the Gas Industry," by G. R. Boreham, B.Sc., A.R.I.C.

Edinburgh, February, 1962:

"Death by Poisoning," by A. C. Hunt, M.D.
"Problems in Criminal Investigation," by Det. Supt. J. K. McLellan, M.A., B.Sc., A.R.I.C.

Glasgow, March, 1962:

"The History of Food Technology," by T. McLachlan, D.C.M., A.C.G.F.C., F.R.I.C., M.I.Biol.

Belfast, June, 1962, jointly with the North of England Section:

Details of the papers read at this meeting are given in the report on the North of England Section.

Glasgow, October, 1962:

"The Chemistry of Wines and Spirits," by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

Glasgow, November, 1962, jointly with the Glasgow Section of the Society of Chemical Industry on Cellulose Ethers:

"Applications of Cellulose Ethers," by F. C. Hall, Ph.D., M.Sc., A.M.I.Chem.E., F.R.I.C.
"Analysis of Cellulose Ethers," by A. F. Williams, B.Sc., F.R.I.C.

Glasgow, December, 1962, jointly with the Chemical Society, the Society of Chemical Industry and the Royal Institute of Chemistry:

"Thoughts on Meat," by E. C. Bate-Smith, M.Sc., Ph.D., M.Inst.R.

WESTERN SECTION—The membership of the Section now stands at 123.

During 1962 there have been 6 meetings of the Section, including the Annual General Meeting. The meetings have all been joint meetings, either with the local sections of the Royal Institute of Chemistry or with a Group of the Society.

The papers presented and discussed were—

January, 1962, Newport, Annual General Meeting, followed by joint meeting with the Cardiff and District Section of the Royal Institute of Chemistry:

"Radioactivity Measurements in Monmouthshire," by G. V. James, M.B.E., M.Sc., Ph.D., F.R.I.C.

March, 1962, Swansea, jointly with the Physical Methods Group and the South Wales Section of the Royal Institute of Chemistry:

"Spectrofluorimetry," by C. A. Parker, B.Sc., Ph.D., F.R.I.C.
"X-ray Fluorescence Analysis," by R. J. Otter, B.Sc., Ph.D.

April, 1962, Newton Abbott, jointly with the South Western Counties Section of the Royal Institute of Chemistry:

"The Technique of Sampling," film introduced by W. T. Elwell, F.R.I.C.
"Sampling of Ore," by G. V. James, M.B.E., M.Sc., Ph.D., F.R.I.C.
"Sampling of Soils and Crops," by B. M. Dougall, M.Sc., F.G.S., A.R.I.C.
"Sampling of Fertilisers, Foods and Feeding Stuffs," by F. W. Marston.
"Sampling of Liquids," by G. J. C. Nash, F.R.I.C.
"Sampling for Atmospheric Pollution," by B. T. Commins, M.Sc., A.R.I.C.
"Sampling of Airborne Dust," by N. M. Potter, M.Sc., Ph.D., F.Inst.F., M.I.Min.Ed., F.R.I.C.

October, 1962, Salisbury, jointly with the Mid-Southern Counties Section of the Royal Institute of Chemistry:

"The Rôle of the Analyst in the Food Industry," by Miss M. Olliver, M.Sc., F.R.I.C.

November, 1962, Gloucester, jointly with the Bristol and District Section of the Royal Institute of Chemistry:

Social evening.

December, 1962, Cardiff, jointly with the Cardiff and District Section of the Royal Institute of Chemistry:

"Gem Stones and Jewels, Natural and Synthetic," by R. C. Chirnside, F.R.I.C.

MIDLANDS SECTION—The membership of the Section is 372, consisting of 353 Ordinary Members and 19 Junior Members. This is a total increase of 17 members during the year. There are 9 Honorary Members of the Section.

Twelve meetings were held during the year: 5 in Birmingham, 2 each in Nottingham and Luton, and 1 each in Coventry, Wolverhampton and Northampton.

The Feigl Anniversary Symposium, honouring Professor Fritz Feigl's 70th birthday, was held at Birmingham University during April, 1962. Professor Feigl, an Honorary Member both of the Midlands Section and of the Society, himself chose Birmingham for this function. The Symposium was most successful. Presentations were made to Professor Feigl by delegations from Russia and Japan, and by the Chairman of the Microchemistry Group on behalf of all Microchemists.

Elwell Award, 1962—This annual award was won by Mr. F. J. Wallace, of Foseco International Ltd., for his paper on "The Determination of Magnesium in Aluminium Alloys by Atomic Absorption Spectroscopy."

The papers presented and discussed were—

January, 1962, Birmingham, on Developments in Gas Chromatography as Applied to Polymers:

"Recent Advances in Technique," by D. H. Desty.
"Gas Chromatography and its Use in Polymer Chemistry," by C. A. Finch.
"The Separation of the Degradation Products of Polymers," by R. S. Lehrle.

February, 1962, Birmingham, jointly with the Midland Region of the Association of Clinical Biochemists:

"Automatic Equipment for the Analytical Laboratory," by G. V. R. Mattock, B.Sc., Ph.D., A.R.I.C.
"Automatic Methods in the Analytical Laboratory," by I. D. P. Wotton, M.A., M.B., Ph.D., F.R.I.C.

February, 1962, Luton:

"Analytical Research," by J. Haslam, D.Sc., F.R.I.C.

March, 1962, Birmingham:

Annual General Meeting

March, 1962, Nottingham:

"Application of Radio-isotopes in Analysis," by D. Gibbons, B.Sc., Ph.D., A.R.I.C.

April, 1962, Wolverhampton:

"The Determination of Boron," by R. H. Biddulph, M.A., B.Sc., Ph.D., and H. J. Cluley, M.Sc., Ph.D., F.R.I.C.

May, 1962, Northampton, jointly with the Physical Methods Group and the Birmingham and Midlands Section of the Royal Institute of Chemistry, on Recent Developments in Semi-conductor Analysis:

"The Spectrographic Determination of Some Impurities in Gallium Arsenide," by J. H. Oldfield, F.R.I.C., and D. L. Mack.
"Radioactivation Analysis of Semi-conductors," by D. Hazelby, A.R.I.C.
"The Determination of Carbon and Silicon in Gallium Arsenide," by A. C. Tyrrell, J. M. Page and D. C. Newton, B.Sc.

May, 1962, Luton, jointly with the Microchemistry Group on The Status of Trace Metal Determinations:

"Ferrous Metals," by B. Bagshawe, F.I.M., M.Inst.F.
"Non-Ferrous Metals," by W. T. Elwell, F.R.I.C.
"Distribution in Soils," by H. H. le Riche, Ph.D.

October, 1962, Birmingham: presentation of papers for the Elwell Award, 1962.

"A Method for the Determination and Characterisation of Organic Bases in Pharmaceutical Preparations," by R. E. King, A.R.I.C.
"The Determination of Fluoride by Complexometric Titration," by M.A. Leonard, B.Sc., Ph.D., A.R.I.C.
"The Determination of Magnesium in Aluminium Alloys by Atomic Absorption Spectroscopy," by F. J. Wallace.

October, 1962, Nottingham:

"The Statistical Approach to Analysis," by D. A. Pantony, T.D., B.Sc., Ph.D., A.R.C.S., F.R.I.C.

November, 1962, Coventry:

"Solvent Extraction," by T. B. Pierce, B.Sc., M.A., D.Phil.

December, 1962, Birmingham, jointly with the Microchemistry Group:

"The Determination of Carbon and Hydrogen in Organic Materials," by Miss A. M. G. Macdonald, M.Sc., Ph.D., A.R.I.C.

MICROCHEMISTRY GROUP—The membership of the Group is now 805, an increase of 48 in the past year.

At the Feigl Anniversary Symposium in Birmingham in April, 1962, the Chairman of the Group, Mr. C. Whalley, presented Professor Feigl with a glass vase on behalf of all microchemists.

During 1962 four Ordinary Meetings of the Group were held: in London on February 23rd. (the Annual General Meeting followed by an Ordinary Meeting for the reading of original papers); in Luton on May 11th (jointly with the Midlands Section); in Dublin from September 21st to 23rd (jointly with the Dublin and District Section of the Royal Institute of Chemistry); in Birmingham on December 14th (jointly with the Midlands Sections). The papers read were—

London:

"The Ultra-micro Determination of Halides at Extreme Dilution," by E. Bishop, B.Sc., A.R.C.S.T., A.R.I.C., and R. G. Dhaneshwar, M.Sc.
"Development of a Simplified Spectroscopic Method for Non-routine Solution Analysis of Trace Metals," by C. P. Cole.
"The Ultra-micro Quantitative Determination of Ammonia by Means of Indanetrione Hydrate," by S. Jacobs, M.Sc., Ph.D., F.R.I.C.

Luton:

Details of the papers read at this meeting are given in the report on the Midlands Section.

Dublin:

"Modern Trends in Small-scale Inorganic Analysis," by Professor T. S. Wheeler, D.Sc., F.R.I.C., F.I.C.I., R. C. Chirnside, F.R.I.C., R. A. Chalmers, B.Sc., Ph.D., and D. J. Hingerty, M.Sc., Ph.D., F.R.I.C.

Birmingham:

Details of the paper read at this meeting are given in the report on the Midlands Section.

Five informal discussion meetings were held in London and one in Dublin. The topics discussed and the speakers who introduced them were:

"Determination of Traces of Copper," introduced by E. I. Johnson, M.Sc., F.R.I.C., and D. B. Adams, B.A., B.Sc.
"Do-it-yourself Ideas in Microchemical Apparatus."
"Ion Exchange in Microchemistry," introduced by J. Pilot, B.Sc., and J. A. R. Genge, M.Sc.
"Differences Between Continental and British Practice in the Determination of Elements in Organic Compounds," by W. Schöniger, Dr.ing.
A Review of all Topics Discussed at these Meetings.
"Modern Trends in Analysis."

PHYSICAL METHODS GROUP—The number of Group members is now 904. This is an increase of 60 since the last Annual General Meeting.

The Group has set up an Atomic Absorption Spectroscopy Discussion Panel, under the Chairmanship of Mr. W. T. Elwell, F.R.I.C., and with Mr. D. Moore as Honorary Secretary, with the aim of arranging discussion meetings on applications of this technique.

During the past year the Group has held five Ordinary Meetings; three were held in London and one each in Swansea and Northampton. The Swansea meeting was held jointly with the Western Section and the South Wales Section of the Royal Institute of Chemistry, the Northampton Meeting jointly with the Midlands Section and the Birmingham and Midlands Section of the Royal Institute of Chemistry, and one London Meeting jointly with the Polarographic Society. The papers read and discussed at the Ordinary Meetings of the Group were—

London, November, 1961:

"Electrochemical Methods in Analysis," by G. W. C. Milner, D.Sc., A.Inst.P., F.R.I.C.

London, February, 1962:

"Some Surface Effects in Electro-analytical Chemistry," by Professor H. A. Laitinen.

Swansea, March, 1962:

Details of the papers read at this meeting are given in the report on the Western Section.

Recent Developments in the Analysis of Semi-conductors—Northampton, May, 1962:

Details of the papers read at this meeting are given in the report on the Midlands Section.

Recent Work in Radiochemical Methods of Analysis—London, October, 1962:

"The Use of Charged Particles for Activation Analysis," by T. B. Pierce, B.Sc., M.A., D.Phil.
"Recent Developments in the Use of Radio-isotopes in Analysis," by T. T. Gorsuch, B.Sc., Ph.D., A.R.I.C.
"Recent Uses of Labelled Reagents in Biochemical Analysis," by J. K. Whitehead, M.Sc., Ph.D., A.R.C.S., D.I.C., A.R.I.C.

BIOLOGICAL METHODS GROUP—During the year the membership of the Group has increased from 316 to 331.

In the year ending on October 31st, 1962, the Group has held, in addition to the Annual General Meeting, three discussion meetings and made one laboratory visit. The papers read and discussed were—

December, 1961, London, Annual General Meeting:

Discussion on "The Assessment of Anti-atherosclerotics," introduced by G. S. Boyd, Ph.D., A.H.-W.C., A.R.I.C.

February, 1962, London:

Discussion on "The Assessment of Antibiotics in Animal Feeds," introduced by G. Sykes, M.Sc., F.R.I.C.

April, 1962, Manchester:

Details of the paper read at this meeting are given in the report on the North of England Section.

ANALYTICAL METHODS COMMITTEE—The progress of work during the year has been maintained. The number of committee meetings (73) showed an increase over that for the previous year (64), although the number of active committees and panels had decreased from 22 to 18. The book of Official, Standardised and Recommended Methods of Analysis, compiled and edited by Mr. S. C. Jolly, is due to appear in print early in 1963: this comprises about 350 pages of recommended methods published in Reports of the Analytical Methods Committee since 1927, together with a comprehensive Bibliography of authoritative methods emanating from countries on both sides of the Atlantic. Also with the printer are recommended methods for determining Trace Elements with Special Reference to Fertilisers and Feeding Stuffs: these are being published as a booklet comprising some 40 pages.

A new departure from the usual type of Report by the Analytical Methods Committee is one prepared by the Sub-Committee on Particle Size Analysis: this is a classification of methods of measurement of particles in the sub-sieve range (*i.e.*, below 76 microns), and it is to be published in *The Analyst* as a Review article. This classification represents the first part of the Sub-Committee's work; the second part is intended to be an appraisement of the principles or techniques of the methods listed in the classification, and it is envisaged that this work, which will necessarily involve experimental tests, will take some considerable time.

Other programmes completed during the year include the preparation of a Report on the Determination of Copper, by the Metallic Impurities in Organic Matter Sub-Committee (now with the printer); a Report on Nitrogen Factors for Beef, by the Meat Products Sub-Committee; a Report on the Determination of Riboflavin in Animal Feeding Stuffs, by the Vitamins (Water-soluble) Panel of the Additives in Animal Feeding Stuffs Sub-Committee; a Report on the Oxygen-flask Method for the Determination of Organically-bound Chlorine in Pesticides and Formulations, prepared by the Chlorine in Organic Compounds Sub-Committee; and a revision of the methods for the Determination of the Capsaicin Content of Capsicum and its Preparations (originally published in *The Analyst* in 1959).

With the completion of most of its programme, the Additives in Animal Feeding Stuffs Sub-Committee has been disbanded: one of its original Panels—on Prophylactics—has now been reorganised as a Sub-Committee in its own right in order to carry on the very lengthy programme originally assigned to it. No new committees or panels have been set up during the year.

LIAISON WITH OTHER SCIENTIFIC ORGANISATIONS—During the year the appointments made were—

Chemical Council:
Dr. D. T. Lewis.

Joint Library Committee, Chemical Society:
Dr. J. G. A. Griffiths.

Parliamentary and Scientific Committee:
Dr. J. H. Hamence.

Royal Institute of Chemistry, Summer School Organising Committee:
Mr. A. N. Leather and Mr. C. Whalley.

International Congress XIX of Pure and Applied Chemistry, 1963, Scientific Committee:
Mr. C. Whalley.

B.S.I. Committees:

Mr. S. A. Price: Chemical Divisional Council.
Dr. C. B. Barrett: Technical Committee on Analysis of Emulsifying Agents etc.
Dr. R. E. Stuckey: Technical Committee on Sampling of Chemical Products.
Dr. R. E. Stuckey: Technical Committee on Bulk Measurement of Chemical Products.
Dr. R. E. Stuckey: Technical Committee on Physical Tests in Chemical Products.
Mr. G. A. Vaughan: Technical Committee on Terminology and Rules for the Expression of Reagent Strengths.
Mr. G. A. Vaughan: Technical Committee on Preferred Ranges of Indicator Solutions.

The Council of the Society thanks all its representatives for the work they have done in the various Committees and at various meetings during the year.

HONORARY TREASURER'S REPORT—The Society's auditors, Messrs. Ridley, Heslop and Sainer, carried out the audit of the Society's accounts, investments, etc., and have provided a balance sheet for the year ended October 31st, 1962. This was submitted to the Finance Committee on Wednesday, December 12th, and approved for submission to Council at their next meeting on Wednesday, January 9th, 1963. Copies of the accounts and balance sheet have been circulated to all members.

The Society continues to possess a strong and vigorous membership, which is still slowly increasing.

It will be remembered that our printers, Messrs. W. Heffer & Sons Ltd., Cambridge, increased their printing charges in 1961 by 10 per cent. for both *The Analyst* and *Analytical Abstracts*. This factor and the reasonable increase in salaries made by the Finance Committee to the permanent staff of the Society have increased expenditure and consequently diminished the excess of income over expenditure. The allocations to the Society's reserves for premises, special publications, etc., were therefore decreased from £1000 to £500. These reserves will be quite heavily depleted in the year 1962–63, by necessary compilation expenses associated with the Annual Index for 1962 and 1963 and also with the Decennial Index covering the first ten years of *Analytical Abstracts.* The compilation costs of these alone will amount to about £1200, exclusive of printing, publication, etc.

No new investments have been made by the Society during the past year, the bulk of the monies having been allowed to accumulate in the Deposit Account at normal rates of interest.

Full details of the Schedule of Investments are given in the balance sheet, the fall in the market value of the Society's holdings being indicative of the financial fluctuations in share values that have affected both this country and the U.S.A. during the past year.

In financial transactions of this nature, the Society is continually advised by its brokers, Messrs. Chance & Co., and there does not appear to be any reason for any particular change in our investment policy. The position of the Society's finances may thus be regarded as satisfactory, although static, but it must be remembered that the Society is not run for profit but for extending those fields of knowledge embracing the science of analytical chemistry. Nevertheless, it is essential that the financial virility of the Society be preserved by the recruitment of new members and by increasing those revenues obtained by the sales, not only of *The Analyst* and *Analytical Abstracts*, but also of those authoritative publications that it is right and proper that the Society should publish.

PROGRAMMES COMMITTEE—Attendance at meetings during the year has been about average, although one evening, that on which Applications of X-ray Fluorimetry were presented, was seriously marred by fog. The element of chance that attends the traditional meetings devoted to the presentation of original contributions has led to a recent decision to abandon this type of meeting and to replace it, for a trial period, by some meetings arranged for the presentation of researches by workers in Universities and Colleges of Advanced Technology. Much time has been devoted during the past year to a consideration of a proposal that the Society should hold a Conference on Analytical Chemistry; the proposal has been accepted and it seems probable at the moment that such a Conference will be held at Nottingham University during the third or fourth week in July, 1965. It is intended that this Conference should be devoted, in the main, to original work rather than to review papers. The scientific content of the meeting will be organised by the Programmes Committee and a local Committee will be formed to deal with its organisation.

THE ANALYST—The introduction with current volume of a white, calendered, paper in place of the former cream matt paper is probably the most noticeable of recent changes made to improve the general appearance of *The Analyst.* Another is the transformation of the old "Notes" section into a section of Short Papers.

The 1962 volume contained 984 pages, 56 more than the record 1960 volume, and 124 more than last year. This increase was required by a slightly greater number (9 more) of papers and notes describing original work, the total being 160, in addition to five Review Papers and four Scientific Reports prepared by various Committees. There has been a sharp increase in the number of Book Reviews (83 as against 59 last year). Circulation has again increased, and 7300 are now being printed of each issue (7000 last year).

Four of the five Review Papers were concentrated in the first half of the year; it has not proved possible to maintain an even flow, and it must be expected that there will be rather greater numbers in some years than in others. Their value to members and non-members alike is emphasised by the continued steady sale of reprints.

Eleven issues of the Bulletin were distributed with *The Analyst* during the year.

During the year Miss E. R. Prince joined the editorial staff in place of Mr. Harris, who has transferred to *Analytical Abstracts* as Assistant Editor.

ANALYTICAL ABSTRACTS—Several changes of staff took place in 1962, the most important being the retirement, on October 31st, of Dr. N. Evers from the Editorship, which he had held since the inception of *Analytical Abstracts* in 1954. He was succeeded by Mrs. H. I. Fisk, with Mr. Brian Harris, formerly Editorial Assistant in *The Analyst* office, as Assistant Editor. Dr. R. E. Essery, who had been working as a part-time assistant since 1959, retired in June.

In spite of these, and other changes, the total number of abstracts published in 1962 was 5564 on 716 pages; this is the highest in any one year since *Analytical Abstracts* began, the previous record being 5522 abstracts in 1960.

Sales have continued to rise and, as a result of a study by a large industrial group in the U.S.A. on a replacement of their own bulletin with *Analytical Abstracts*, we have now received an order from them for 150 subscriptions in 1963.

In view of this order it was decided that we should try to extend our advertising in the U.S.A., on an exchange basis. Of the several journals approached, favourable replies were received from *Applied Spectroscopy*, *Journal of the Association of Official Agricultural Chemists* and *Cereal Science Today*. Although it is against the policy of the *Journal of Biological Chemistry* to enter into exchange agreements, they have offered to insert an occasional advertisement, free of charge, when convenient to them. The equivalent advertisements from America will appear in *The Analyst*, this being a more suitable medium than *Analytical Abstracts*.

The Decennial Index will be due in 1964, and it has been arranged that Dr. N. Evers will prepare the matter for the Subject section.

Certain changes were made in the constitution of the Editorial Committee. Mr. B. A. Ellis retired from the Chairmanship in July, but agreed to remain on the Committee as an ordinary member, and was succeeded by Mr. B. S. Cooper; Mr. A. G. Jones, Imperial Chemical Industries Ltd., Plastics Division, was co-opted in October as an additional member to deal with the organic chemistry field; and Dr. N. Evers, on his retirement from the Editorship of *Analytical Abstracts*, has agreed to remain on the Committee as an ordinary member.

An abstractors' meeting, followed by a lunch, was held in March. About 40 regular abstractors attended, and many problems, both editorial and abstracting, were discussed.

A. J. AMOS, *President.*
R. E. STUCKEY, *Honorary Secretary.*

Address of the Retiring President

A. J. AMOS, O.B.E., B.Sc., Ph.D., F.R.I.C.

(Delivered after the Annual General Meeting, March 8th, 1963)

The Society Yesterday, To-day and To-morrow

WE have reached a stage in the life of our Society when more than ever before in its history we should be forward-looking. The potentialities for extension of the scope of its interests, for expansion of its membership and for enhancement of its status are great, but, if they are to be brought to fruition, our outlook must be more a welcome for the future than a pride in the past. We must give as much thought and supervision to a long-term policy as we do to the planning of our yearly programmes. Before this can be done, we must have clear in mind the direction and the scope of the progress we wish to achieve; we must be agreed upon our goal before we can plan ahead. In this address I shall leave with you my thoughts upon how and to what end we should direct our efforts in the hope that they may prove to be a stimulus that will lead to the preparation and implementation of a planned policy for the future. But although my main concern is to look ahead, I am mindful of the precept of Lord Halifax that—

> "The best way to suppose what may come is to remember what is past."

The birth certificate of our Society is to be found in the *Chemical News* for August 14th, 1874, where it is reported that 27 analysts, who were dissatisfied with the Report of a Parliamentary Committee on the Adulteration Act of 1872, met together at a Cannon Street hotel and agreed that "an Association of Public Analysts be formed for the purpose of mutual assistance and co-operation." The name selected for the Association was "The Society of Public Analysts." The reason that prompted the formation of this Association, its inaugural constitution and its adopted title justify the conclusion that the initial interest of our Society was not analytical chemistry, but the analysis of food and drugs. That this was so is clear from the objects of the Society, which were published a few months later. These were—

(*i*) To promote and maintain the efficiency of the laws relating to adulteration.

(*ii*) To promote, and as far as possible to secure, the appointment of competent Public Analysts.

(*iii*) To improve the processes for the detection and quantitative estimation of adulteration and to secure uniformity in the statement of the results by holding periodical meetings for the reading and discussion of original papers on chemical and micro-chemical analysis, especially with reference to the detection of adulteration.

When a quarter of a century had passed, the Rules of the Society were amended so that membership was not restricted to "analysts in practice," but was open to anyone who had a genuine interest in analytical chemistry. Eight years later, when the Society was incorporated, opportunity was taken to extend its title and to broaden its objects in recognition of the "other analytical chemists" who were then within its ranks. Nevertheless, another 5 years passed and the Society was 38 years old before precedent was broken and the Presidential Chair was occupied by a representative of the "other analytical chemists."

Reference to the volumes of *The Analyst* published in that and the other years of the fourth decade of the Society's existence provides supporting evidence that the interests of the Society were becoming less sharply focused on food and drugs and were coming more into line with its revised objects, which were "to encourage, assist and extend the knowledge and study of analytical chemistry and of all questions relating to the analysis, nature and composition of natural and manufactured materials." Critical surveys of advances in methods of analysing cellulose and rubber were commissioned for *The Analyst* and the subjects of original papers in the journal included dyestuffs, paper, turpentine, mineral oils, rubber, disinfectants, metallic ores and alloys, detergents, coal, gases, blood stains and documents.

Diversification of its interests material-wise continued to increase as the Society veered numerically towards a Society of Other Analytical Chemists and Public Analysts. At the same time, because of the progressive discovery of new analytical tools, a cross segregation of members based on methodology tended to develop. Eventually this trend reached a stage that warranted official recognition and support, and these were provided in 1943 by the decision of Council to permit the formation within the Society of Groups for the furtherance of specialised branches of chemical analysis. In less than 2 years, three Groups were in existence—a Microchemistry Group, a Physical Methods Group and a Biological Methods Group.

At the time the formation of the first Group was imminent, which fell in the 70th year of the Society's existence, the number of papers dealing with food and drugs appearing in any volume of *The Analyst* was little more than one-third of the total. The preponderance of papers on analysis in other contexts tended to become even greater as papers read at meetings of the three active Methods Groups began to flow into the journal, because very few of them dealt with food and drugs. This pronounced shift of emphasis in the themes of the papers in the Society's journal was paralleled by a change in the structure of its membership; by the time the Society was 75 years old its "other analytical chemists" outnumbered its Public Analysts by more than 15 to 1. From a small group of Public Analysts absorbed in the problems of food and drug analysis the Society had grown into a large band of chemists whose collective interests covered the fundamental principles of analytical chemistry, methodology, the application of analytical techniques in a wide range of contexts other than food and drugs, and the teaching of analytical chemistry.

The name of the Society, which in any event was too long and cumbersome, was no longer apposite, and, indeed, was misleading. Some progressively minded members of Council who subscribed to Bernard Shaw's dictum that "all progress is initiated by challenging current conceptions and executed by supplanting existing institutions" began to press for a change of title, but they met considerable opposition; they found it easy enough to "challenge current conceptions" but a very uphill task to "supplant existing institutions." However, eventually good sense prevailed and at the close of 1953 the Society became "The Society for Analytical Chemistry" and entered the fold of learned societies.

Some Public Analysts took the view that with this change they were losing their birthright, whereas their emotion should have been pride in the fact that the vision and the efforts of their enthusiastic forbears who founded an Association in 1874 and nurtured it in its early years had culminated in a learned Society of nearly 2,000 members that catered for all aspects of theoretical and applied analysis. I am sure that those early Public Analysts to whom we owe so much—men like Redwood, Muter, Allen, Hehner and Dyer, to mention but a few—would have been proud indeed had they had a foresight of the outcome of their labours.

With this change of name, the Society of yesterday became the Society of to-day. We are now in the last year of our first decade as a learned Society and it is an opportune time to review our achievements and our policies—to decide whether our resources, our plans and our activities have been so directed that we are serving analytical chemistry in general and our members in particular to the best of our capabilities.

It is much to the credit of the Society that when British Chemical Abstracts were discontinued, an event that occurred about the time the Society changed its name, Council decided to repair the loss to analytical chemists by publishing monthly a journal of abstracts of analytical papers. This was particularly a laudable action because it called for additional and specialised staff and it was undertaken at a time when the Society was experiencing an annual loss on its journal. Council's faith in this venture to serve analytical chemistry was vindicated, and to-day the sales figure for *Analytical Abstracts* is over 7500. The international reputation of these abstracts, which are the only ones relating to analytical chemistry that can be purchased independently of abstracts of other branches of science, is deservedly high, but I believe that neither in status nor sales has this publication yet reached its zenith.

At the time the Society of yesterday became the Society of to-day it was making an annual loss of about £2000 on *The Analyst,* and within 3 years the deficit in the annual accounts of *Analytical Abstracts* was a similar sum. These losses were made good—as were the publication losses of other scientific societies—by grants from the Chemical Council, but the situation was a frustrating one. Before the Society could hope to progress, it had to become self-supporting, and it achieved this target in 1958, being the first of the four beneficiary Societies to dispense with the aid of the Chemical Council. It has consolidated its financial position in the intervening years, and, although it has been and will again be faced with unavoidable rises in expenditure, I am confident that we shall never again go back on the dole.

Another innovation made since the Society changed its names is the Programmes Committee. Before its appointment, the paper-reading meetings were planned by the Publication Committee and with few exceptions they consisted of papers selected from those submitted for publication in *The Analyst*. This practice served well enough yesterday, but in the face of the quickening tempo of scientific research its retention would have made the Society a historian instead of a pioneer of analytical developments.

The planning of the Programmes Committee has been based upon the sound premise that in selecting topics for meetings of the Society priority should be given to those subjects that are currently of widespread interest. In place of the old type of meeting in which several already submitted papers on widely divorced subjects were read—a type of meeting well suited to the earlier days of the Society of Public Analysts—it favours the meeting at which invited specialists present papers on a common theme, particularly the principles and practice of a modern analytical technique. Reference to the 1962–63 programme of the Society reveals that four of the six paper-reading meetings are of this type, the subjects, for each of which a series of papers was commissioned, being polarography, X-ray fluorescence, particle size analysis and magnetic resonance. The 1961–62 programme had a similar pattern, three of the five meetings having as their respective themes, the oxygen flask combustion technique, new analytical reagents and the application of infrared spectroscopy to analytical problems. In giving the programmes of the Society this new look, the Committee did not at first eliminate the older type of meeting, but in the light of later experience it has decided not to retain it as a regular feature.

The meeting arranged for April resembles the old type of meeting in that the programme embraces a number of topics, but there the similarity ends; the papers have not been chosen from those submitted to *The Analyst*, but will be contributions from research students in universities and colleges of advanced technology. This is a new venture and if it is successful it might well be repeated in future years. Even if these meetings for research students attract only the relatively small audiences that tended to become characteristic of our traditional multi-subject meetings, there is a good case for their continuance; they will have a missionary impact by bringing the Society to the notice of potential members, whereas at the old type of meeting we only preached to the converted.

Because of the trend of advance in applied analysis, the topic of a one-theme meeting will more often than not be a physical method of analysis. Not infrequently the method warrants periodical discussion over a number of years because its devoted band of followers introduce modifications into the technique, make improvements in an instrument or discover new applications of the analytical procedure. However, there is a limit to the number of meetings the parent Society can hold each session, and it may well be that the only way it can keep pace with the advance of analytical chemistry is to relegate the organisation of more meetings to specialist panels.

The creation of a Sub-Group, Panel—call it what you will—to provide a forum for the discussion of a new specialised analytical technique may have an insurance value by arresting the formation of what has been called a "splinter group," since the best way of ensuring that the exponents and adherents of a new technique conduct their discussions and reviews under the aegis of the Society is to give them a niche within our constitution. I am sure that Council took a step in the right direction when last year it approved the precedent of permitting non-members of the Society to join the recently formed Atomic Absorption Spectroscopy Discussion Panel. By thus fostering in a practical manner the interests of specialists outside the Society, we effect a liaison that may in the course of time bring some of them into our ranks. Had we been sufficiently forward-looking in this respect in the past the Society might have become the centre for discussions and reports of activities and progress in a newer technique by specialists whose desire to foregather urged them to form organisations of their own.

As long ago as 1884 the Council then in office appointed a Committee to examine the various methods of milk analysis in use and to report upon their respective accuracies. Forty years later, the need for standardisation of analytical methods had extended to other fields of work and had become sufficiently pressing to prompt the Council to appoint a standing Committee to advise when and in what direction a study of the applicability and reliability of competitive methods was desirable. This was the forerunner of the present Analytical Methods Committee. Within a short time, the standing Committee of 1924 had advised and Council had approved the formation of two working Sub-Committees, one to deal with the analysis of condensed and dried milk and the other with essential oils. Last year, its offspring, the Analytical Methods Committee, was supervising 18 Sub-Committees and Panels, each of which was either guiding or conducting research on specific analytical problems.

This notable expansion of the work of the original Standing Committee was made possible by an action taken soon after we became the Society of to-day, a progressive action of "supplanting existing institutions." This was an appeal to industry to provide the funds that would enable the Analytical Methods Committee to maintain a paid Secretariat. Until then, not only the collaborative analytical work but all the necessary ancillary secretarial work had been performed free of charge by members of the Sub-Committees and Panels. They were all busy men whose professional duties had first call on their time and accordingly the consideration of the outcome of a practical exercise and the issue of a final report were often seriously delayed because the associated not inconsiderable volume of paper work could not be done quickly.

Following industry's avowed interest in and financial support for the work it was doing, the Analytical Methods Committee widened its activities and published a "Bibliography of Standard, Tentative and Recommended or Recognised Methods of Analysis," a book of 34 sections that contained well over 6000 references. Encouraged by the sale of 1500 copies of this book, the Committee has produced a second edition on more ambitious lines; it is wider in scope and reproduces in full instead of by reference all the Committee's recommended methods. This book, which bears the title "Official, Standardised and Recommended Methods of Analysis" was published at the beginning of March. Between these two editions, the Analytical Methods Committee published a book of recommended methods for the analysis of trade effluents, based on an investigation made jointly with the Association of British Chemical Manufacturers, and, in a few weeks, will have on a sale a book on the determination of trace elements.

The parent Society also has added to what are termed "other publications" in the Annual Accounts by initiating what will become a series of Monographs. It is planned that these Monographs shall be books for the bench rather than for the reference library. The series began with "Methods for the Analysis of Non-Soapy Detergent Products" and this will shortly be followed by "The Determination of Sterols."

Supplementary publications edited by the parent Society or by its Analytical Methods Committee can and undoubtedly will enhance the status and benefit the finances of the Society, but we must never lose sight of the fact that *The Analyst* and *Analytical Abstracts* are its life blood. We cannot afford, therefore, to be complacent about sales of *The Analyst* and *Analytical Abstracts*, but must periodically review our publication policy. The wisdom of so doing is proved by the fact that in its recent report the specially appointed Analyst Development Committee saw fit to make 30 recommendations.

In comparing the Society of to-day with the Society of yesterday I have made no reference to number of employees, size of office accommodation, annual income and expenditure, number of pages in *The Analyst*, and the like because retrospective comparison of these and other material items are not a true measure of progress. Our concern should be whether we are doing more than we did to stimulate and to sustain the march of analytical chemistry, whether the contributions we may make to this end take heed of the ever-widening horizons of this branch of science, and whether we give adequate coverage to each of the multitudinous interests of those concerned with the teaching, the theory and the practice of analysis. And particularly we should ask ourselves whether we cater too much for the expert and the experienced and serve too little the embryo and newly fledged analysts, because there is truth in Franklin Roosevelt's observation that the test of progress "is not whether we add more to the abundance of those who have too much; it is whether we provide enough for those who have too little."

Looking back as we have done at the Society of yesterday enables us to gauge the soundness and the success of the plans adopted as measures of development. That we have made progress is beyond doubt: now the scope of the Society's interests extends, as it should do, over the whole realm of analytical chemistry viewed from the standpoint of techniques or contexts; the meetings of the parent Society are supplemented by meetings organised by specialist Groups and by new Sections, thereby providing greater opportunity for members to keep pace with new work; the old type of meeting comprising papers on unco-ordinated subjects has been replaced by a single-subject meeting that attracts an audience with a keen interest in all the papers in the programme; the work of the Analytical Methods Committee has been intensified and accelerated and has earned the interest and financial support of industry; the programme of each Society meeting is judiciously planned and planned well in advance by a specially appointed Committee instead of remaining a matter of chance; finances have been put on a sound basis; and the Society has undertaken the task of making available throughout the world abstracts of analytical literature. In the light of these and other developments it cannot be denied that to-day the Society, through its meetings, through its journals, through its "other publications" and through its Analytical Methods Committee, is serving analytical chemistry and analysts to better effect than did the Society of yesterday.

Having looked from to-day into the past, it is time to turn to the future. What of the Society of to-morrow? Its control will not be in our hands, but the success that attends the efforts of those who will be in charge may be much influenced by the course we pursue —what they reap will spring from what we sow.

Our first endeavour must be to ensure that in our meetings and in our journal we give complete coverage in the field of analytical chemistry no matter how greatly or how rapidly the field extends. To achieve this, we must maintain a keen, active and well-balanced Programmes Committee. The primary function of this Committee, which, I believe, may well become the most important of the Society's Committees, will be to keep constant watch for promising new techniques, for new applications of existing techniques, and for industrial and legal developments that create new problems for the analyst, and then to arrange meetings at which specialists will speak on these subjects. A secondary duty of the Committee might well be to remedy the absence from *The Analyst* of papers on a subject that is of widespread interest or of growing importance by arranging a Symposium on the subject.

The possibility and desirability of arranging joint meetings with other organisations should also be frequently reviewed by the Committee. Such meetings not only assist the Society to extend its sphere of influence, but may engender interest in it and thereby initiate recruitment among those who have never enquired what it has to offer because they do not regard themselves as analysts. There are many in this category and we should not neglect any measure that will attract them to our ranks. A chemist who is occupied in making quantitative determinations of one or more constituents of a parent material is still an analyst, even if the identification and separation are performed by an instrument and his final measurement that completes the assay involves only the taking of a reading on this same or an ancillary instrument. He may be termed a physicist or a physical chemist, but he is just as much an analyst as his predecessor who made the same determinations by classical analytical procedures, and we should take positive steps to bring to his notice the fact that there is a place for him in our Society.

There is need also to forge a link between the Society, perhaps through specialised groups, and organised bodies of those who are interested in the construction and performance rather than the application of specialised analytical tools. Such liaison benefits both bodies; it enables the analyst to understand better the performance, the sources of error, the specificity and the sensitivity of the instrument and it suggests to the instrument specialist possible new analytical uses of the tool.

During the investigation made by the Analyst Development Committee, it became clear that despite our graduation from a professional body to a learned Society, *The Analyst* had not acquired the status of a learned journal throughout the academic world. It is understandable that in days gone by the channel of publication sought for a paper on some fundamental principle of analysis was one of the recognised learned journals rather than one devoted mainly to papers on food and drugs with special reference to adulteration. The justifiable hope that this attitude would be abandoned in the light of the change in the status of the Society and in the contents of its journal was not realised. No longer do papers on food and drugs predominate in *The Analyst*, but academic research workers are still loth to see their papers surrounded by papers on "applied" analysis. This is not the time nor place to delineate upon the line of demarcation between or the relative importances of applied analysis and what has been variously qualified as fundamental, pure or basic analysis, but the issue is clear. Our policy for the future must be to eradicate misconception about the Society and its interests and to preach the interdependence of pure and applied analysis until *The Analyst* is accepted in academic circles as an appropriate journal for the analytical papers that at present they send elsewhere.

In the first place, we may have to go out and seek such papers, relying upon personal appeal and persuasion by members of Council and of the Publication Committee. If these efforts are successful, other papers will follow and the inflow will become self-expanding. But we can do more than this. We can plan to bring the Society more frequently and more closely to the notice of academic institutions. Notices of meetings on notice boards can help, but a closer contact is required; we need to bring the Society itself and not merely records and announcements of its activities into the universities and technical colleges. We are making a start in this direction in the meeting that is arranged for April at the Chelsea College of Science and Technology, but we should plan others that are not designed specifically for post-graduate students.

April's meeting brings the Society to the notice particularly of young analysts still in training—potentially Society members of to-morrow. It is better that they should have first-hand knowledge of the Society before and not after they go into industry or teaching, and we should strive to make this type of meeting a regular feature of our annual programme. We should look upon these meetings as "bread upon the waters" and accordingly not be disheartened if at first they do not attract large audiences.

If we achieve what should be our aim—to have the Society of to-morrow recognised as all-embracing in its interests in the field it serves—then its meetings and its journals will be considered to be appropriate for contributions upon not only basic analytical procedures, the application of analysis in specific contexts and the potentialities of specialised analytical tools, but also upon the theory, history, philosophy and teaching of analytical chemistry. As this aim approaches realisation, a venture worthy of consideration would be the publication of two journals rather than a considerably enlarged *Analyst*. *The Analyst* would be devoted to papers on analytical problems and procedures particular to the application of analysis in specific contexts, and a new journal, which might be entitled "The British Journal of Analytical Chemistry" would be the site of theoretical and practical papers on fundamental analytical chemistry and papers on the history, philosophy and teaching of analytical chemistry. In my opinion, this would be a development worthy of mature consideration. I realise that it would involve various administration and financial problems, but I believe that they could be solved without great difficulty.

Analytical Abstracts has made and will continue to make the Society more widely known, and now produces an income in excess of the cost of production. These are sound, albeit material, reasons why the publication should have a major call upon the Society's resources, but there is an ethical reason why we should be prepared to make special efforts to ensure its continuation. Having voluntarily undertaken the task of providing this service for analysts, we are under a moral obligation to do all that is within our power to give it permanency. And our pride as a learned Society and our desire to stimulate the advancement of analytical chemistry should preclude lack of financial support or inadequate supervision of administration ever causing *Analytical Abstracts* to fall short of the high standard they have attained.

The existing Group structure of the Society had much to commend it when it was introduced 20 years ago, but its retention unchanged in the Society of to-morrow might become a sign of old fashioned thinking. To-day, very many analysts who are unconversant with filter sticks and other specialised and elegant equipment dear to the heart of the earlier microchemists are working with samples or determining amounts much smaller than those that were the topics of discussion in the early meetings of the Microchemistry Group. As micro-analysis becomes progressively more commonplace, the retention of a major specialist Group devoted to microchemistry will be difficult to justify. The wisdom of retaining the Biological Methods Group as a major Group is arguable for a different reason. Biological methods are still a specialised branch of analysis, but because it is so very specialised its application is limited and advances of note are few and far between. Accordingly, the stage has been reached when the Group Committee finds itself at a loss each year how to build a programme. The reverse holds good in the Physical Methods Group. Developments in and extension of the applications of physical methods have been so great that physical techniques warranting discussion far exceed the number of Group meetings that can be held. Moreover, the methods and their uses are of such general interest that many of the meetings of this Group might well be meetings of the parent Society and *vice versa.* I believe, therefore, that the days of the existing Groups are numbered; the interests of two of them are insufficiently specialised and those of the other too highly specialised for them to remain as major Groups in the Society of to-morrow—the segregation of interests would be too artificial and unrealistic. Rather do I see a series—and a changing series—of Panels or Discussion Groups that will look after the interests of specialists and ensure that the main and subsidiary meetings of the Society between them give ample coverage to the latest developments and trends in analytical chemistry. Some of these Panels or Groups will be longer lived than others, but whenever a stage is reached at which it becomes an onerous task to scrape together sufficient papers to build what has previously been a customary programme, the function of a Group should be relegated to a watching brief for a new discovery or a resurgence of interest in its specialised field.

Thought will have to be given to the place and function of the Analytical Methods Committee of the Society of to-morrow. It has been suggested that the work performed by this Committee and its Sub-Committees and Panels, although in keeping with the interests and the activities of the Society of yesterday, does not come within the purview of a learned Society. This is not my view. I believe it right and proper for a learned Society concerned with analytical chemistry to initiate and sponsor investigations designed to evaluate or to improve the reliability of existing or to devise new methods of analysis. But it is also my belief that the investigations it sponsors should extend over a wide area of analytical chemistry and embrace both fundamental and applied problems. The work of the Analytical Methods Committee in the Society of yesterday and to-day has not done this; it has been limited to a narrow field and, in view of the too slowly disappearing impression that the Society's interests are still only those of the Public Analysts, it is unfortunate that the narrow field is that of food and drugs.

Restriction of all but two or three of the Committee's investigations to food or drugs has not been a matter of choice, because the investigations are prompted by requests put to the Committee, not infrequently by other bodies. The reasons why requests for investigations have not come to the Committee from industries other than those concerned with food or drugs should be investigated in the hope that the situation can be rectified, because the scope of the Committee's researches in the Society of to-morrow must be much wider. It must be much wider not only to preclude its giving a misleading picture of the Society's interests and status, but also to safeguard the essential financial support it receives from industry. It is unrealistic to expect organisations whose business is in petroleum, heavy chemicals, engineering, rubber, metals and the like to be willing to subsidise heavily researches that rarely extend beyond problems in the analysis of food and drugs. If these industries cannot or do not submit analytical problems to the Committee, then the scope of the investigations should be widened by initiating or sponsoring research of a fundamental nature. I am convinced that if in the Society of to-morrow the work of the Analytical Methods Committee continues to be confined within the present narrow limits, the scheme will have an adverse effect upon the reputation of the Society and, moreover, is likely to become but a shadow of its former self through lack of financial support.

In planning for the Society of to-morrow, we should not be content to fix our sights at the national level. The Society has 255 overseas members representing 47 nationalities and non-member subscriptions to the Society's journals come from over 90 countries. This should serve as an incentive for us to formulate our policy for the future with not only the national but the international reputation of the Society in mind. To-day, many of the non-member subscribers to *The Analyst* and *Analytical Abstracts* know the Society only as a name that appears on those journals; we should not rest until they and all other analysts throughout the world recognise it as an authoritative body in the field of analytical chemistry that is contributing significantly to the advancement of that branch of science. One means to this end is to make the Society periodically the focal point of a gathering of analysts from all parts of the world. I welcome, therefore, the approval by Council of a plan to hold a Conference on analytical chemistry in 1965. I believe that with dedicated determination on the part of Council, with an enthusiastic Conference Committee and with the experience of the highly successful conferences that have been held at Oxford, St. Andrews, Edinburgh and Birmingham, this meeting can achieve a success that will be an assurance that the goal of international acceptance of the Society as an organisation of high standing in the field of analytical chemistry is realistic and attainable.

The universe of analytical chemistry is expanding at a fascinating speed; so great has been progress in the recent past that looking ahead we can expect to see, in the words of Alexander Pope, "New distant scenes of endless science arise." The opportunities before us are therefore great, but unless our outlook is continuously anticipatory, the time will come when we shall begin to lose the status we have won. We should be constantly on the watch for developments that attract increasing attention or are likely to do so because of their potentialities so that at an appropriately early stage they can be the subjects of papers in our meetings or journals. If we wait until a new trend in analysis has been established before we provide a platform for it, we shall be too late. On more than one occasion in the past we have thus lost a great opportunity, and specialist groups that might have been part and parcel of the Society have arisen as independent bodies. This should never happen again —the Society should be in the van of the advance and not a camp follower.

In bringing to a close this review of the prospects that lie before the Society and steps we can take to bring them to fruition, I would remind you of those lines from Lowell—

"New times demand new measures and new men;
The world advances, and in time outgrows
The laws that in our fathers' day were best."

Let us hope then that the succession of members through whose hands will pass the formulation and the implementation of policy will be men of vision, men who look from the plains of to-day to the heights of to-morrow. And let us be firmly resolved not to be bound by the shackles of tradition, but ever ready to prune, to reconstruct and to explore.

Anniversary Dinner

IN the evening following the Annual General Meeting, a Dinner to celebrate the eighty-ninth anniversary of the Society was held, by kind permission of the Court of Assistants of the Grocers' Company, at Grocers' Hall, Princes Street, E.C.2. The members and guests, numbering 155, including all the living Past Presidents of the Society, were received by the President, Dr. A. J. Amos, O.B.E., F.R.I.C. and Mrs. Amos. The President afterwards took the Chair at the Dinner.

The Guests of the Society and of the President included The Right Honourable the Lord Todd, F.R.S., (Past President of The Chemical Society) and Lady Todd; The Honourable Mr. Justice Lloyd-Jacob, M.A., D.C.L., (Chairman of the Analytical Methods Trust); E. Le Q. Herbert, Esq., B.Sc., F.H.-W.C., F.R.I.C., M.I.Chem.E., F.Inst.P., F.Inst.F., (Past President of The Royal Institute of Chemistry) and Mrs. Herbert; S. I. Levy, Esq., Q.C., M.A., Ph.D., F.R.I.C., and Mrs. Levy; D. D. Moir, Esq., M.Sc., F.R.I.C., (President of The Association of Public Analysts) and Mrs. Moir; Mrs. B. Lamb, B.Sc., F.R.I.C., (Chairman of The Polarographic Society) and Mr. Lamb; M. A. T. Rogers, Esq., B.Sc., Ph.D., F.R.I.C., (Research Controller, Imperial Chemical Industries Ltd.) and Mrs. Rogers.

The Loyal Toast was proposed by the President.

Lord Todd, proposing the toast of The Society for Analytical Chemistry, recalled his undergraduate course in inorganic analysis—gravimetric determinations, by text-book methods, of elements in simple solutions, starting with silver nitrate and progressing through the classical qualitative analytical groups of metals. Perhaps this hardy beginning had led him away from analytical into other kinds of chemistry; it certainly had led him to appreciate the skills required of practising analysts. But unless a proper training was given, which included arousing an interest in the subject, the profession of analytical chemistry was likely to be short of recruits. This was not simply a task for the Universities or Colleges of Technology, but was one that the Society was particularly well fitted to tackle. He concluded with a tribute to *Analytical Abstracts*, and to the Society for starting when *British Abstracts* had stopped and thereafter developing *Analytical Abstracts* into the world's foremost single-subject abstracting journal.

Dr. Amos replied by recalling that in his Presidential Address he had dwelt on the Society's past, present and future. The Society was as concerned as Lord Todd at the difficulty of getting sufficient analysts, and it intended to show that it had much to offer to those engaged in teaching analytical chemistry, doing fundamental research into the subject and working to solve the analytical problems of industry, commerce and legislation. To this end it was planning closer liaison with the Universities and Technical Colleges, and was reorganising its specialist panel system to cater for all advances in technique as soon as they appeared. Success in this depended on those who gave their time to serve as Members of Council and as Honorary Officers, both of the parent Society and of the Sections and Groups. There was no lack of such voluntary workers and, in paying tribute to them, he felt that the Society's success was assured.

Dr. D. T. Lewis, the Government Chemist and Honorary Treasurer of the Society, proposed the toast of The Guests. Besides the representatives of other Societies, there were present Mr. Justice Lloyd-Jacob—a Bernard Dyer Memorial Medallist of the Society as well as Chairman of the Analytical Methods Trust—and Dr. S. I. Levy, an eminent Queen's Counsel, who had served in the Ministry of Munitions in the first World War and was Assistant Director of the Ministry of Supply in the second. Also all the living Past-Presidents of the Society were present, making this a unique occasion.

Dr. S. I. Levy, Q.C., in reply, alluded to the days when he and Dr. Amos had been Officers of the Chemical Club. He had left chemistry to make himself a career in the Law. He was particularly pleased that Mr. Justice Lloyd-Jacob, Her Majesty's Special Judge appointed to deal with Patent cases, was present. It had been his privilege and pleasure to appear many times before his Lordship. On behalf of all the guests he thanked the Society for its hospitality.

The proceedings concluded with Dr. Amos calling on Dr. D. C. Garratt, the incoming President and Chairman of the Analytical Methods Committee, and investing him with the Presidential Badge. Dr. Garratt presented Dr. Amos with a replica of the Society's Badge to wear as Past President.

Applications of Infrared Spectroscopy

Part X.* The Zeisel Determination of t-Butoxyl Groups, and the Anomalous Reactions of t-Butylphenols†

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Zeisel determinations on t-butoxyl compounds give non-quantitative and variable results. t-Butyl iodide decomposes thermally to isobutene, the equilibrium involved being affected by the reaction variables and by the addition of phenolic compounds. The over-all effect is therefore particularly complex for aromatic t-butoxyl compounds, since phenolic compounds are formed within the reaction medium as de-alkylation occurs.

Results are presented showing that more satisfactory analyses can be obtained when hydrobromic acid is used in place of hydriodic acid. t-Butyl bromide suffers $\ngtr 2$ per cent. decomposition to isobutene when boiled under reflux with constant-boiling hydrobromic acid; moreover, this decomposition is reproducible under given reaction conditions, and correction factors can therefore be applied. Since t-butoxyl compounds are de-alkylated almost as quickly in hydrobromic acid as in hydriodic acid, the reaction periods required are not significantly longer; the period required varies from 2 to 3 hours, and is dependent on the nature of the sample.

Boiling under reflux with constant-boiling hydrochloric acid offers a method of differentiating between true t-butoxyl compounds and those t-butyl compounds that react anomalously in hydriodic and hydrobromic acids.

THE utilisation of t-butyl and t-butoxyl compounds has increased greatly in recent years, *e.g.*, in antioxidants,[1,2] perfumery chemicals,[3] free-radical reactions,[4,5] graded oxidants[6,7] and in chromatographic separations.[8] The relatively easy removal[9,10] of t-butyl and t-butoxyl groups makes them useful in reaction intermediates[11] and as protective groups in syntheses, *e.g.*, of peptides.[12] Steric effects,[9] rearrangements[13] and instability[14,15] are factors that combine with the property of ease of removal to complicate the functional analysis of t-butoxyl groups. t-Butylphenols react anomalously in Zeisel determinations,[16,17] and some of the attendant analytical difficulties have been indicated.[18]

Only a few papers have discussed the application of the Zeisel reaction to butoxyl compounds in general. Of these, only two—so far as we are aware—have quoted results for the tertiary isomer. Houghton and Wilson[19] reported, without comment, a recovery of only 18·8 per cent. of the theoretical yield of t-butyl iodide from t-butyl alcohol; under different reaction conditions, Kirsten and Nilsson[20] obtained 60 to 70 per cent. recoveries, and stated that "tertiary butanol appears to give a fairly stable volatile iodide, although the reproducibility of recovery is not good."

It has long been known that t-butyl iodide is unstable at its boiling-point (103° C), the equilibrium—

$$C_4H_9{}^tI \rightleftharpoons C_4H_8 + HI$$

being established.[21] Some decomposition must therefore occur in Zeisel determinations (compare Campbell and Chettleburgh[16]), in which the reaction temperature is 127° C. In view of the discrepancies between the recoveries reported,[19,20] a spectroscopic[22] study of the recovery of t-butyl iodide from reflux in hydriodic acid was undertaken in an attempt to improve the accuracy of determining t-butoxyl groups.

It became clear that the use of hydriodic acid was analytically unsatisfactory when it was found that: (*i*) variation of the reaction conditions gave recoveries of t-butyl iodide ranging from 19 to 80 per cent.; (*ii*) under standardised reaction conditions, the yields of t-butyl iodide were affected by the presence of phenolic compounds in the reaction medium.

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t-Butoxyl compounds react rapidly[23,24] with aqueous hydrobromic and hydrochloric acids, and t-butyl bromide and chloride are more stable thermally than is the iodide; the possibility of basing analytical reactions on boiling under reflux with those acids was therefore investigated.

EXPERIMENTAL

COMPOUNDS—

(*a*) Samples of t-butyl alcohol[25] and t-butyl halides conforming to literature description were obtained by redistillation under reduced pressure of reagent-grade commercial samples. Since t-butyl iodide quickly develops a dark colour, small amounts were redistilled daily. Isobutene was prepared by dehydration (with concentrated sulphuric acid) of purified t-butyl alcohol.

(*b*) *t-Butyl ester*—t-Butyl 3,5-dinitrobenzoate was prepared; the specimen conformed to literature description.

(*c*) *t-Butyl ethers*—t-Butyl phenyl ether, t-butyl-*p*-tolyl ether and t-butyl-1-naphthyl ether were prepared by Grignard reactions with t-butyl perbenzoate[26,27]; the specimens gave satisfactory elemental analyses (Weiler and Strauss, Oxford). Dark colours developed on storage, and these specimens were redistilled under reduced pressure as required.

(*d*) *t-Butylphenols*—Samples were supplied by Dr. R. L. Williams, Messrs. Kodak Ltd. and Messrs. I.C.I. (Dyestuffs Division) Ltd. Liquids were purified by redistillation. Most of the samples, however, were low-melting solids not readily purified by recrystallisation; these were purified by zone-melting.

APPARATUS, REAGENTS AND PROCEDURE—

These have been described,[28,29] together with details of (*i*) the technique for trapping volatile reaction products and (*ii*) the infrared vapour-phase method for their subsequent identification and determination. Particular care is necessary when transferring the contents of the trap to the gas-cell; t-butyl iodide decomposes so readily that direct warming of the trap over a flame is inadvisable. Satisfactory results were obtained by immersing the trap in water at 80° to 90° C, the sodium chloride cell windows being suitably protected (with plastic covers) during this operation.

A slight reaction occurred between t-butyl halides (particularly the iodide) and the sodium chloride cell windows, so that the windows "fogged" much more quickly than usual. The validity of calibration curves had therefore to be checked more frequently than in previous investigations.

USE OF SOLID SCRUBBERS—

Aqueous solutions hydrolyse t-butyl halides to t-butyl alcohol; hydrolysis of the iodide occurs extremely rapidly.[30] It is therefore essential (compare Campbell and Chettleburgh[16]) to use a solid scrubber in determinations of t-butyl halides. Soda asbestos[31] has given satisfactory results throughout our studies.

RESULTS

EXPERIMENTS WITH CONSTANT-BOILING HYDRIODIC ACID—

(*a*) *Rate of reaction of t-butoxyl compounds*—Zeisel determinations were conducted on t-butyl alcohol, t-butyl 3,5-dinitrobenzoate and t-butyl-1-naphthyl ether under standard conditions. The conditions were: volume of hydriodic acid, 6 ml (sp.gr. 1·70, pre-conditioned[28]); nitrogen flow rate, 6 to 8 ml per minute; weight of phenol added, 30 mg. Sample weights yielding 2 to 4 mg of t-butyl iodide were taken. The yields of t-butyl iodide at the reaction times stated were as shown in Table I. Burwell, Elkin and Maury[32] have already commented on the fact that ethers are not always less reactive than alcohols.

(*b*) *Recovery of t-butyl iodide*—Samples of t-butyl iodide (in small weighing bottles fitted with ground-glass stoppers—see Anderson and Duncan[28]) were placed in the Zeisel reaction flask; the recovery from boiling under reflux in hydriodic acid was investigated, the reaction conditions being the same as those outlined in (*a*) above. The maximum recovery varied from 58 to 80 per cent.; about 80 per cent. of the total recovery in each determination distilled within 20 minutes. For fixed weights of samples of t-butyl iodide, small variations in recovery

TABLE I: YIELD OF t-BUTYL IODIDE FROM DIFFERENT COMPOUNDS

Compound		Yield of t-butyl iodide (as percentage of theoretical) After reflux for 1 hour	After reflux for 2 hours	After reflux for 3 hours
t-Butyl 3,5-dinitrobenzoate		76·0	80·8 (max.)	—
t-Butyl-1-naphthyl ether		40·5	66·6 (max.)	—
t-Butyl alcohol	1st determination	42·5	48·7	56·6 (max.)
	2nd determination	39·5	45·0	60·9 (max.)

resulted when: (*i*) the volume of hydriodic acid was decreased from 6 to 1 ml, (*ii*) the flow rate was varied from 4 to 12 ml per minute and (*iii*) the weight of phenol was varied from 0 to 100 mg. Little variation in the rate of recovery was found when the temperature of the condenser water was increased (compare Belcher, Fildes and Nutten[33] and Inglis[34]).

(*c*) *Production of isobutene*—In all these determinations some isobutene was produced, the sum of the molar recoveries of t-butyl iodide and isobutene accounting for the t-butyl iodide taken.

A time - recovery experiment with 2,6-di-t-butyl-4-methoxyphenol in which Campbell and Chettleburgh's[16] experimental conditions were used gave results agreeing well with those reported[16]; boiling under reflux for 1 hour gave the theoretical yield of methyl iodide, together with isobutene and a yield of t-butyl iodide that, calculated as methyl iodide, gave an apparent methoxyl content of 21 to 22 per cent. The ratio of the molar yields of isobutene and t-butyl iodide was, however, constant over the whole reaction period, *e.g.*, boiling under reflux for 10 minutes gave approximately 70 per cent. of the total yield of isobutene and also approximately 70 per cent. of the total yield of t-butyl iodide. This does not support Campbell and Chettleburgh's implication[16] that the isobutene results from decomposition of some t-butyl iodide that does not distil in the earlier stages of the reflux period.

The molar ratio of isobutene to t-butyl iodide (*i.e.*, the extent of decomposition of the t-butyl iodide) was also much greater than in any of our previous experiments. It was suspected that this resulted from the changes made in the reaction conditions in order to duplicate Campbell and Chettleburgh's experiments.[16] These workers, in testing scrubber effects, determined the total apparent methoxyl content of 2-t-butyl-4-methoxyphenol under four different reaction conditions, and found four different values ranging from 18·06 to 22·56 per cent. This range was confirmed when these experiments were repeated with a solid scrubber. Changes in reaction conditions, and not scrubber hydrolysis effects, therefore cause the variable results. This effect was further investigated as described below.

(*d*) *Variation in yield of t-butyl iodide with reaction conditions*—In a series of experiments, a constant weight of t-butyl-4-hydroxyanisole (mixed 2 and 3 isomers) was allowed to react under different conditions; these are shown, together with the yields of t-butyl iodide obtained, in Table II. Further experiments showed that cresols and other phenolic compounds caused similar variations in the results. The conjoint addition of other solubilisers, such as propionic anhydride and hypophosphorous acid, further complicated the effect.

Other experiments indicated that phenolic compounds, formed in the reaction medium during the de-alkylation reaction, contributed to the decomposition of t-butyl iodide. (1) t-Butyl-4-hydroxyanisole (5 mg) was allowed to react in hydriodic acid (6 ml), with no added phenol. The recovery of t-butyl iodide was 80 per cent., in agreement with the result

TABLE II: YIELD OF t-BUTYL IODIDE FROM 5-mg SAMPLES OF BUTYLATED HYDROXYANISOLE UNDER DIFFERENT REACTION CONDITIONS

Nitrogen flow rate, ml per minute	Hydriodic acid (sp.gr. 1·70) used, ml	Phenol added, mg	Yield (as percentage of theoretical)
4	1	30	19
4	6	30	51
6 to 8	1	10	53
6 to 8	6	0	80
6 to 8	6	10	72
6 to 8	6	30	62
6 to 8	6	60	50
12 to 15	1	30	45
12 to 15	6	30	73

in Table II. (2) The reaction medium was boiled for a further 4 hours to eliminate all traces of alkyl iodides, and the mixture was then cooled. A further 5 mg of sample and 30 mg of phenol were added; the recovery of t-butylphenol was 52 per cent., compared with 62 per cent. (see Table II) for the corresponding "straight" reaction with 30 mg of phenol. (3) The reaction medium was again boiled for 4 hours, and then cooled; a further 5 mg of sample and 30 mg of phenol were added. The recovery of t-butyl iodide was then only 42 per cent., compared with 50 per cent. for 60 mg of phenol in Table II. Thus the effect of the total phenol added (60 mg) was apparently augmented by the sum (approximately 6 mg) of the weights of phenolic compounds formed in determinations (1) and (2).

EXPERIMENTS WITH CONSTANT-BOILING HYDROBROMIC ACID—

(*a*) *Recovery of t-butyl bromide*—When 6 ml of hydrobromic acid, 30 mg of phenol and a nitrogen flow rate of 6 to 8 ml per minute were used, the recovery of samples (2 to 5 mg) of t-butyl bromide after boiling under reflux for 1 hour was 90·7 per cent. and, after 2 hours, 98·4 per cent. (maximum yield). An equilibrium of the form

$$C_4H_9{}^tBr \rightleftharpoons C_4H_8 + HBr$$

must exist,[35] but under the stated reaction conditions (reflux temperature 115° C) the extent of decomposition to isobutene does not exceed 2 per cent. Indeed, only traces of isobutene were detectable in the infrared spectrum of the reaction products; some polymerisation[35] of isobutene may occur. The recoveries of t-butyl bromide were reproducible and were not strongly influenced by small changes in reaction conditions or in the amounts of phenol added.

(*b*) *Rate of reaction of t-butoxyl compounds*—With use of the same reaction conditions as in (*a*) above, the rate of evolution of t-butyl bromide from some t-butoxyl compounds was determined. The results are shown in Table III. The reaction time required varies from 2 to 3 hours, depending on the compound being analysed. When these results are corrected by +1·6 per cent. (the percentage loss of t-butyl bromide during recovery), only the results for two of the ethers are slightly low; this may well reflect the state of purity of these specimens.

(*c*) *The anomalous reaction of t-butylphenols*—Some t-butylphenols were boiled under reflux in constant-boiling hydrobromic acid for 2 to 3 hours. Nearly quantitative yields of t-butyl bromide were produced. Such compounds cannot therefore be distinguished from t-butoxyl compounds by this reaction.

TABLE III: YIELD OF t-BUTYL BROMIDE FROM t-BUTOXYL COMPOUNDS

Compound	Yield of t-butyl bromide (as percentage of theoretical)		
	After reflux for 1 hour	After reflux for 2 hours	After reflux for 3 hours
t-Butyl alcohol..	95·4	98·3 (max.)	—
t-Butyl 3,5-dinitrobenzoate	97·0	98·6 (max.)	—
t-Butyl phenyl ether	86·5	91·1	96·2 (max.)
t-Butyl-p-tolyl ether	86·8	93·3	95·6 (max.)
t-Butyl-1-naphthyl ether	90·8	93·6	97·5 (max.)

EXPERIMENTS WITH CONSTANT-BOILING HYDROCHLORIC ACID—

Under the reaction conditions specified in "(*a*) Recovery of t-butyl bromide" above boiling under reflux with constant-boiling hydrochloric acid for 2 hours gave the results listed below.

(*a*) Recovery of added t-butyl chloride was nearly quantitative (>98 per cent.) (compare Kistiakowsky and Stauffer[36]).

(*b*) t-Butyl alcohol and t-butyl 3,5-dinitrobenzoate gave 98 per cent. of the theoretical yields of t-butyl chloride.

(*c*) t-Butyl ethers gave 60 to 65 per cent. of the theoretical yield of t-butyl chloride.

(*d*) The reactions of the t-butylphenols were—

(*i*) No t-butyl chloride formed—
    2,4-di-t-butylphenol;
    5-methyl-2-t-butyl-4,6-dinitroanisole;
    4-t-butylphenol.

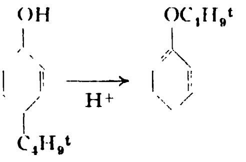
(*ii*) <10 per cent. of t-butyl chloride formed—
5-methyl-2-t-butylphenol;
t-butylated-4-hydroxyanisole (mixed 2 and 3 isomers);
2,6-di-t-butylphenol.

(*iii*) <20 per cent. of t-butyl chloride formed—
2,4-dimethyl-5-t-butylphenol;
3-methyl-4,6-di-t-butylphenol.

CONCLUSIONS

Boiling under reflux with constant-boiling hydrobromic acid is a satisfactory method of analysis for t-butoxyl groups. Under the reaction conditions described, decomposition of t-butyl bromide does not exceed 2 per cent., and the appropriate correction factor can be applied to the analytical results. The reaction period varies from 2 to 3 hours, depending on the compound being analysed. When the infrared method of determination[22] is being used, prolongation of the reaction period is not critical, although this might be inadvisable for volumetric or gravimetric determinations of the t-butyl bromide. Boiling under reflux with constant-boiling hydrobromic acid does not distinguish between true t-butoxyl compounds and t-butylated phenols.

Boiling under reflux with constant-boiling hydrochloric acid, however, offers a method of making this distinction. The yields of t-butyl chloride vary from 60 to 98 per cent. for t-butoxyl compounds, and from 0 to 20 per cent. for the range of t-butylphenols studied. It is possible that a rearrangement[27] of the form—



occurs in acid solution, the extent of the rearrangement depending on the concentration of acid and the nature of the substituent groups and substitution pattern in the phenolic compound.

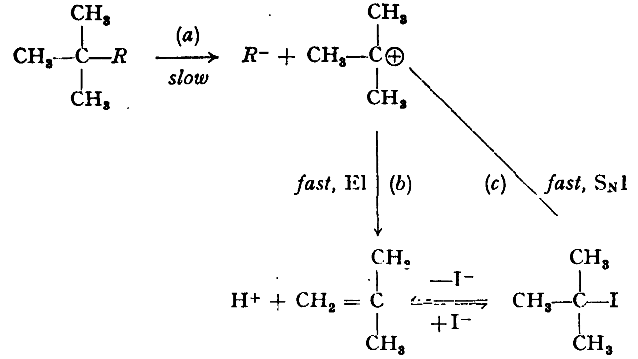
The results presented show clearly that boiling under reflux with hydriodic acid does not give a satisfactory analytical reaction for t-butoxyl groups. The equilibrium—

$$C_4H_9{}^tI \rightleftharpoons C_4H_8 + HI$$

is clearly dependent on the ratio of sample weight to volume of acid used, on the flow rate of scavenging gas and on the amounts of phenolic compounds added as solubilisers or formed within the reaction medium during the de-alkylation reaction.

Our results indicate that production of isobutene does not increase markedly in the latter stages of a determination; there is no extensive decomposition of undistilled portions of t-butyl iodide. Two effects must be distinguished. In the recovery of t-butyl iodide, the yield varied from 58 to 80 per cent. and was not strongly dependent on changes in flow rate or on the addition of phenolic compounds. The rate of distillation was rapid (approximately 80 per cent. in 15 minutes), and the recovery of added t-butyl iodide appears to be mainly dependent on the thermal decomposition equilibrium. In the formation of t-butyl iodide from t-butoxyl compounds, however, several striking differences are apparent. (*a*) The relative yields of t-butyl iodide vary much more widely (19 to 81 per cent.); (*b*) the rate of distillation (now dependent on the rate of formation) is much slower (approximately 50 per cent. in 1 hour); (*c*) the relative amounts of t-butyl iodide and isobutene formed are strongly dependent on the reaction conditions, and, particularly, on the presence of added compounds.

Mechanisms of the reactions of t-butyl compounds have been extensively studied,[37,38] and the relative stability of the t-butyl carbonium ion is well known. Olefine-forming elimination reactions involving butyl compounds proceed via competitive S_N1 and E1 unimolecular reactions, in which the rate of formation of the carbonium ion (step (*a*) below) is rate-determining.



It appears that the relative extent to which reactions (*b*) and (*c*) occur in Zeisel determinations is dependent on the reaction conditions and additives employed.

We are grateful to Dr. R. L. Williams, Ministry of Aviation, Waltham Abbey, Essex, Messrs. Kodak Ltd., Kirkby, Lancs., and Messrs. I.C.I. (Dyestuffs Division) Ltd. for providing specimens of t-butylphenols. We thank the P.C.S.I.R., Karachi, for granting study leave and financial assistance to one of us (S.S.H.Z.).

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A Cryoscopic Method for assaying Pyridine

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A method is described suitable for the routine assay of pyridine. Water present in the original sample or absorbed during the assay has an abnormal effect on the freezing-point, and errors arising from this source are overcome by applying a correction based on the water content of the sample at the end of the test.

The depressions of the freezing-point of pyridine caused by additions of up to 6 moles per cent. of α-picoline, β-picoline, γ-picoline, 2,6-lutidine and benzene have been measured; these substances include all the major impurities likely to be present in pyridine derived from natural or synthetic sources. For each of the compounds added the depression of the freezing-point was linear over the range examined, and the values of the cryoscopic constant were identical within the limits of error of the experimental procedure.

Within the range of our experiments, therefore, the accuracy of the method is not dependent on the composition of the sample, and the single equation presented relating freezing-point to purity can be used with confidence for the assay of pyridine from natural or synthetic sources.

Results of an inter-laboratory co-operative programme for determining the precision of the method are reported.

IN 1959 Adey and Cox[1] described a method, based on solution temperature in aqueous potassium chloride, for determining small amounts of α-picoline in pyridine. If the assumption is made that α-picoline and water are the only contaminants of commercial pyridine, the procedure affords a precise and accurate measure of the purity of this chemical. However, there is always the possibility that other bases may be present. It is known, for example, that one synthesis of pyridine also yields β-picoline, and we have found that the depression of the solution temperature of pyridine is 1·09° C per 1 per cent. w/w of β-picoline fraction compared with 0·70° C per 1 per cent. w/w of α-picoline present. Further, the effect of benzene, which may be used in the azeotropic dehydration of pyridine, is some 10 times as great as that of α-picoline (6·9° C per 1 per cent. w/w of benzene).

Later, a simple cryoscopic method for assaying β-picoline, γ-picoline and 2,6-lutidine was reported.[2] During this work it was found, as might be expected, that the bases formed ideal solutions in so far as there were no significant differences in the depression of the freezing-point of a given base by several different basic impurities. Thus, for example, the separate depressions of the freezing-point of γ-picoline produced by 1 mole per cent. of each of four different impurities (α-picoline, β-picoline, 2,4-lutidine and 2,6-lutidine) ranged from 0·537° to 0·550° C. It was concluded, therefore, that the freezing-point was a better criterion of purity in that it was less sensitive to the nature of the basic impurities present. The adaptation of the method to pyridine posed various problems, among them the necessity to work at a much lower temperature, about −42° C, than with the picolines, and the inability to use a mercury thermometer at this temperature, but these were solved more easily than was at first expected. Thermometers having the mercury - thallium eutectic as the indicating liquid were readily obtained to special order, and unqualified assistants found no difficulty in working with a cooling bath at −48° to −50° C.

EXPERIMENTAL

PURIFICATION OF BASES—

Samples of β-picoline, γ-picoline and 2,6-lutidine were purified by slow fractional freezing as described by Biddiscombe, Coulson, Handley and Herington,[3] the process being repeated until no further rise in freezing-point was observed.

The freezing-points of the purified bases were within 0·1° C of the values reported by these authors for the pure bases. This we considered adequate for our purpose.

The freezing-points of pyridine (−42° C) and α-picoline (−67° C) were too low for the same procedure to be adopted with the apparatus at our disposal. These bases were therefore purified by high-efficiency fractional distillation of the commercially pure products.

The pyridine submitted to this treatment was assayed at 99 per cent. purity by the solution-temperature method.[1] The α-picoline used had a boiling range of 1·7° C (drop to dry).

A glass fractionating column was used; this was 5 feet long by 1 inch internal diameter. It was packed with 1/16-inch × 1/16-inch Dixon gauze rings and surrounded by an air jacket with electric heaters for temperature compensation. Take-off was controlled by an electronically operated vapour-dividing still head and boil-up was measured with a conventional boil-up meter interposed between the still and the column.

Two litres of α-picoline were placed in the still and distilled at a boil-up of 1000 ml per hour and a reflux ratio of 50 to 1. The first 20 per cent. by volume of distillate was rejected and the next 60 per cent. by volume collected for use. Analysis by gas - liquid chromatography showed no compounds other than α-picoline.

The pyridine was distilled in a similar way. The final product had a freezing-point of —41·8° C. Its purity as determined by the solution-temperature method was 99·7 moles per cent.

DEPRESSION OF FREEZING-POINT BY WATER—

Because of our experience with the picolines and 2,6-lutidine, which we found to be extremely hygroscopic,[2] no attempt was made to prepare solutions of known amounts of water in pyridine. Instead a suitable amount of water was added to pyridine, and the freezing-point of the mixture was determined. The concentration of water present at the end of the determination was measured by the Karl Fischer method.

In one series of experiments the mixtures used contained approximately 0·05, 0·1, 0·2, 0·4 and 0·6 per cent. of water in pyridine. Their freezing-points and water contents were measured by the procedure described below.

The determinations were repeated on a second series of samples containing 0·02 to 0·9 per cent. of water. As with the picolines, a graph of water content against depression of freezing-point was linear within the range examined.

DEPRESSION OF FREEZING-POINT BY BASIC IMPURITIES AND BENZENE—

Weighed amounts of each impurity in turn were added to weighed amounts of pyridine (see Table I). The water contents of all the substances used were determined, and the molar concentration of dry impurity in the total dry mixture was calculated for each mixture in turn. The freezing-points were converted to a dry basis by determining the water content at the end of the test and applying the appropriate correction from the graph described above. Water contents were kept as low as possible in order to minimise the correction, and were in every instance less than 0·1 per cent.

TABLE I

CONCENTRATIONS OF IMPURITIES IN PYRIDINE

Impurity	Range of concentration, moles per cent.
α-Picoline	0·0 to 4·9
β-Picoline	0·0 to 5·3
γ-Picoline	0·0 to 5·9
2,6-Lutidine	0·0 to 3·9
Benzene..	0·0 to 4·9

METHOD

APPARATUS—

The freezing-point apparatus is of the conventional type and consists of an inner test-tube, 150 mm × 25 mm, fitted concentrically by means of a cork inside a wider tube, 150 mm × 40 mm, that acts as an air-jacket. The inner tube is closed by a cork fitted with a suitable thermometer (see below) and a glass stirrer. The stirrer is a glass rod about 3 mm in diameter bent at its lower end into a loop at right angles to the axis of the rod. This loop is of suitable diameter (about 18 mm) to surround the stem of the thermometer and move easily up and down the inner tube. The thermometer is centrally placed in the cork and so positioned that the bottom of its bulb is about 1 cm above the bottom of the inner test-tube.

The cooling liquid is contained in a Dewar jar, internal diameter about 100 mm, to minimise absorption of heat from the atmosphere.

Apparatus for the determination of water content by the Karl Fischer method is also required.

THERMOMETERS—

Two thermometers have been used. Both were calibrated for 100-mm immersion, subdivided to 0·1° C and had N.P.L. certificates or the maker's Works Certificates quoting corrections with an error of 0·1° C for temperatures above —45° C.

(*i*) Range —46° to —34° C and —0·5° to +0·5° C, made by Short & Mason Ltd., London.

(*ii*) Range —55° to —25° C, made by H. J. Elliott Ltd., E-Mil Works, Pontypridd, Glamorgan.

PROCEDURE—

Place in the Dewar jar an amount of cooling mixture such that, when the apparatus is assembled, the level of liquid in the jar is at least as high as the level of the sample in the inner test-tube. Adjust the temperature of the mixture, immediately before use, to between 6° and 8° C below the expected freezing-point of the sample. A suitable cooling mixture can be prepared from solid carbon dioxide and ethanol. Place approximately 25 ml of the sample to be tested in the inner test-tube. Fit the stirrer and thermometer in the inner test-tube, and pre-cool the sample, with stirring, to about 5° C above the expected freezing-point. Rapidly dry the outside of the test-tube, and fit it centrally inside the air-jacket already in place in the cooling bath. Stir gently and continuously, and read the thermometer at 30-second intervals (estimate the temperature to 0·01° C). When the temperature has fallen to the expected freezing-point, introduce a seed crystal as rapidly as possible, and continue the test. (The seed crystal can be conveniently introduced by raising the stirrer to its highest extent, without removal of the cork from the inner test-tube, and depositing a crystal from a glass rod as low as possible on it. The stirrer is then replaced in the liquid and stirring is continued). The freezing-point corresponds to the first set of four consecutive readings during which the temperature remains constant. If supercooling occurs, the constant temperature will be observed after the temperature rise. A temperature rise of 1° C is the maximum permissible; if it exceeds this value, repeat the determination on a fresh portion of the sample.

Record the observed freezing-point, F, corrected for any scale error of the thermometer.

Remove the inner test-tube, complete with thermometer and stirrer, without delay, and heat rapidly, with stirring, until the temperature rises to between 14° and 16° C. By pipette, preferably with use of a pipette filler, withdraw 20 ml of sample, and determine its water content (per cent. w/v).

Calculate the corrected freezing-point, F_0, for the dry substance by adding an amount 2·30 *w*, where *w* is the water content (per cent. w/v).

Calculate the purity of the dry sample, P_0, from the expression—

$$P_0, \text{ mole per cent.} = 100 - \frac{(-41{\cdot}62 - F_0)}{0{\cdot}594}$$

Alternatively, if the impurities are known to be picolines, the percentage w/w purity, P_w, can be calculated from—

$$P_w, \text{ per cent. w/w} = 100 - \frac{(-41{\cdot}62 - F_0)}{0{\cdot}505}$$

If many samples are to be tested, it is much more convenient to prepare graphs from these expressions and also of 2·30 *w*.

RESULTS

The depression of the freezing-point of pyridine by water was calculated by the method of least squares for each series of mixtures.

The equations for the regression lines are—

(*i*) $F_0 = -41{\cdot}82 - 2{\cdot}321\,w;\ s = 0{\cdot}091$

(*ii*) $F_0 = -41{\cdot}77 - 2{\cdot}284\,w;\ s = 0{\cdot}074$

where F_0 = observed freezing-point, °C, corrected for scale errors of the thermometer,

w = water content at the end of the test, per cent. w/v, and

s = standard error of the regression coefficient.

The effects of basic impurities and benzene on the freezing-point of pyridine are shown in Table II.

TABLE II

DEPRESSION OF FREEZING-POINT OF PYRIDINE BY OTHER BASES AND BENZENE

Impurity	Depression of freezing-point (θ) caused by 1 mole per cent. of impurity,* °C	Standard error of θ	Number of observations
α-Picoline	0·5824	0·012	8
β-Picoline	0·5963	0·016	8
γ-Picoline	0·5880	0·015	6
2,6-Lutidine	0·5534	0·022	7
Benzene	0·5613	0·013	6

* Mixture consists of 99 moles per cent. of dry main component and 1 mole per cent. of dry impurity.

DISCUSSION OF RESULTS

A statistical examination of the values of the depression of the freezing-point (θ) and its standard error, S.E. (θ), for the bases in Table II, showed that the values of θ did not differ significantly from each other. The over-all regression coefficient for bases and its standard error were therefore calculated and, in addition, the over-all regression coefficient for the three picolines, *i.e.*, excluding the value for 2,6-lutidine. The results of the calculations are shown in Table III.

TABLE III

VALUES OF THE OVER-ALL REGRESSION COEFFICIENTS

	θ	S.E. (θ)	Number of observations
All bases	0·5954	0·0070	23
Picolines only	0·5943	0·0055	18

Although the regression line for 2,6-lutidine has the lowest value of slope, the inclusion of the results for this base increases rather than decreases the regression coefficient for the combined results. The difference between the values of the slopes is, however, without significance; it arises from the reduction in weight given to the freezing-point of pure pyridine (which is common to all the regression lines) when the several sets of results are combined.

The value for benzene ($\theta = 0{\cdot}5613$) was not included in the calculation of the over-all coefficient, since benzene is not a usual contaminant of pyridine, but may occur, *e.g.*, through malfunctioning of an azeotropic dehydration unit. The results show that the presence of small amounts of benzene will not vitiate an assessment of the purity of a sample of pyridine from its freezing-point. The method described here does not therefore suffer from the disadvantages of the solution-temperature method in this respect.

Biddiscombe, Coulson, Handley and Herington,[3] who used iso-octane as the impurity, obtained a value of 0·607° ± 0·017° C for the depression of the freezing-point of pyridine by 1 mole per cent. of impurity. The results given here agree closely with this figure. The same workers reported a freezing-point of −41·55° ± 0·05° C for 100 per cent. pyridine. A later personal communication from Dr. Herington gave −41·62° ± 0·0° C as the best estimate for the pure base, and this value has been used in the expression on p. 361 for calculating the molar purity from the freezing-point.

It will be noted that the denominator of this expression is 0·594, that is, the value of θ corresponding to the picolines only. Although there are theoretical reasons for preferring this value to the over-all value of 0·595, the difference is, as stated above, without significance, and substitution of the latter value does not give rise to a significant difference over the range of purity under discussion. A freezing-point of −44·59° C, for example, corresponds to 5·00 moles per cent. of impurity when $\theta = 0·594$ and to 4·99 moles per cent. when $\theta = 0·595$.

This method has been adopted by the Standardisation of Tar Products Tests Committee in its handbook[4] and the Tar Bases Panel of that Committee has carried out an inter-laboratory co-operative programme to determine the precision of the method. They found a repeatability of 0·20° C, corresponding to 0·34 moles per cent. of pyridine, and a reproducibility of 0·50° C, corresponding to 0·85 moles per cent. of pyridine. These are 95 per cent. confidence limits, and in the long run of properly conducted tests the majority of pairs of results will differ by much less than these amounts. The repeatability and reproducibility are expected to improve further as the operators become more familiar with the test.

Results obtained in the laboratories of the Midland Tar Distillers show good agreement between the solution-temperature[1] and freezing-point methods, as the results in Table IV show.

TABLE IV

COMPARISON OF SOLUTION-TEMPERATURE AND FREEZING-POINT METHODS

Sample No.	Solution temperature (S.T.), °C	Freezing-point (F.P.), °C	Purity calculated from— S.T., % w/w	F.P., % w/w	Difference S.T. − F.P.
1(*a*)	26·64	−41·85	99·49	99·54	−0·05
1(*b*)	26·72	−41·78	99·60	99·68	−0·08
1(*c*)	26·58	−41·86	99·40	99·52	−0·12
2(*a*)	26·48	−42·10	99·26	99·05	+0·21
2(*b*)	26·40	−42·08	99·14	99·09	+0·05
3	26·15	−42·16	98·79	98·93	−0·14
4	26·11	−42·19	98·73	98·87	−0·14
5	26·27	−42·14	98·96	98·97	−0·01
6	26·45	−42·03	99·21	99·19	+0·02
7	26·56	−41·98	99·37	99·29	−0·08
8	26·19	42·19	98·84	98·87	+0·07

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Determination of Particulate Acid in Town Air

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A method for measuring particulate acid in town air by titration was investigated and found to be suitable. Particulate matter of the air was collected by filtration, and the amount of acid determined by immersing the collected sample in a known excess of 0·01 N sodium tetraborate in de-ionised water at pH 7 and titrating back to pH 7 with 0·01 N acid. The particulate acid in the air of the City of London appeared to be mainly sulphuric.

VARIOUS workers have studied the acidity of urban air since regular observations of atmospheric pollution began in 1913. They all recognised that the main gaseous acid, apart from the carbon dioxide, in air was sulphur dioxide, but they also identified sulphuric acid in the suspended matter.

Ellis[1] measured the total acid in London air by titration after absorbing both sulphur dioxide and particulate acid in hydrogen peroxide; he determined the sulphur dioxide alone by an iodine method and the concentration of particulate acid by subtraction. Goodeve[2] used sintered-glass discs to filter out the acid, and Coste and Courtier[3] devised a technique in which the acid droplets were made to grow before collection. The general conclusion at that time was that the amount of particulate acid present in urban air was extremely small in comparison with the amount of sulphur dioxide. With the advent of "smog" in Los Angeles, interest in acid droplets was revived, and Mader, Hamming and Bellin[4] used washed filter-papers to collect acid droplets there. All these investigators found significant amounts of acid only in fog.

In the London fog of December, 1952, sulphur dioxide but not particulate acid was measured; this episode revived interest in the subject.

When suspended matter in London air is collected by impaction on glass slides coated with gelatin containing thymol blue and viewed under a microscope, strongly acidic droplets can be seen as pink spots.[5] This method is, however, only qualitative, and, of the various procedures for determining acid, the titration of air solids collected on a filter-paper was found to be the most suitable.

METHOD

DETERMINATION OF PARTICULATE ACID—

Samples for analysis are collected on 4·25-cm circles of Whatman No. 1 filter-paper held in a Perspex holder so that a 1-inch circle of the filter-paper is exposed. Air-flow rates of up to 30 litres per minute are used, and samples are usually taken over periods of up to 6 hours. At times of high pollution, a one hour sample representing 1 cu. metre of air was adequate for analysis.

The method involves titration of filter-papers to pH 7. A solution of bromothymol blue in de-ionised water is prepared by adding 4 ml of a 0·1 per cent. solution of the indicator in alcohol to 100 ml of de-ionised water. To this solution sufficient 0·01 N sodium tetraborate is added to make it a stable apple-green colour (pH approximately 7). After the outer unexposed edges of the filter-paper have been cut off, the sample is cut into two exactly equal portions, one portion being added to 1 to 2 ml of the solution and titrated with 0·01 N sodium tetraborate to the original green colour. A similar beaker containing the same volume of the solution is kept as a control, since this liquid absorbs any sulphur dioxide from the air and slowly changes colour. During titration the solution is agitated by vigorous swirling, and it is found that the end-point is reached after about 5 minutes. This end-point is shown by a stable green colour identical to that of the control solution.

The amount of acid indicated by this method is too low, since some of the acid reacts with water-insoluble bases present in the sample. The true amount of acid is found by adding a known excess of 0·01 N sodium tetraborate (at least 0·1 ml more than the amount indicated above) to 1 to 2 ml of the pH 7 solution and then immersing the second portion of the filter-paper in it and titrating the excess with 0·01 N sulphuric acid. Then the concentration of acid (calculated as sulphuric acid) in μg per cu. metre of air = $98{,}000 \times \frac{\mathrm{N}v}{V}$, where

N = normality of sodium tetraborate (0·01 N),

v = equivalent volume (in ml) of 0·01 N sodium tetraborate used to neutralise the acid during back titration of half the sample filter-paper, and

V = volume of air sampled (cu. metres).

STORAGE OF SAMPLES—

Samples become neutralised when left in air, and they should therefore be titrated as soon as possible after collection. Samples are neutralised more readily indoors than outdoors and only slowly when sealed in polythene bags. Tests have shown that, outdoors, losses of acid varied between 0 and 30 per cent. over 5 days. For the same period indoors, losses up to 70 per cent. occurred owing no doubt to greater amounts of ammonia being present. Samples kept in polythene bags showed a loss of about 50 per cent. over a period of one year.

DISCUSSION AND JUSTIFICATION OF METHOD

Many substances other than sulphuric acid are present in air. Possible interferences in the method were examined and are discussed below.

INTERFERENCE BY ACIDIC GASES—

Carbon dioxide, nitrogen dioxide and sulphur dioxide are all present in polluted air. Carbon dioxide is an extremely weak acid, is not appreciably absorbed by filter-paper and therefore does not interfere with the method. Nitrogen dioxide, even if it were absorbed by filter-paper during collection, is insufficiently absorbed by water to affect the measurement of sulphuric acid. To assess the interference by sulphur dioxide, 200 p.p.m. of the gas at high humidity was passed for 2 hours through a filter-paper loaded with various amounts of smoke collected from the air; no additional sulphuric acid was detected on the filter-papers, and thus sulphur dioxide can be assumed not to interfere significantly with the method.

INTERFERENCE BY BASIC GASES—

When particulate acid can be detected in town air, small amounts of free basic gases (ammonia and amines) can be found also. The co-existence of these two pollutants can be explained by the acidic particles being covered by carbonaceous, tarry or other solid material that hinders neutralisation with ammonia. In order to find out whether ammonia neutralises the acid during sampling, it was removed before the acid was collected. This was done by impinging the air approximately 2 cm above the surface of the concentrated sulphuric acid, a flow rate of 20 litres per minute being used. Preliminary experiments showed that ammonia gas mixtures of between 0·2 and 10 p.p.m. in air are absorbed with efficiencies of between 70 and 95 per cent., and that, when a prepared mist of sulphuric acid (2·5 mg per cu. metre, mass median diameter 0·50 μ), was used, losses in the impinger did not exceed 6 per cent. Acidic particles of this size are found in the polluted air of London during fog; at other times the acid particles are smaller and the loss by impingement would be even less than that found in these experiments (there are also larger acidic particles, but these represent only a small fraction of those found in the polluted air of London). Several samples of acid were collected on filter-papers after ammonia had been removed in this way. These filter-papers were titrated and the results compared with those from samples collected without prior removal of ammonia. Sixteen pairs of samples were collected, each for 8 hours, in the City of London. Comparison of individual results showed in some instances small differences, but the average of the sixteen samples was 3·96 μg per cu. metre of acid, after the removal of ammonia, compared with 3·67 μg per cu. metre of acid for normal collection. This small difference (7·5 per cent.) between the results indicates that there is not enough ammonia present in the air of the City of London to invalidate this method of collecting the sulphuric acid.

INTERFERENCE BY OTHER PARTICULATE ACIDS—

Particulate acids other than sulphuric may be present in town air. These are hydrochloric, nitric, phosphoric, sulphurous and nitrous acids; these acids may be wholly or partly caught on a collection filter-paper. Sulphuric acid can be efficiently filtered from air.[4] Only extremely small amounts of nitrate, phosphate, sulphite and nitrite can be detected in samples collected on filter-paper, and the concentrations of the acids corresponding to these anions must therefore be extremely small. Solids collected from the air of the City of London contained from 0·2 to 2·4 mg of hydrogen ion per g (measured by titration). The chloride content varied between 15 and 150 mg per g, and if this chloride were all hydrochloric acid it would correspond to between 0·4 and 4·2 mg of hydrogen ion per g of solids, so that all the indicated acidity could be due to hydrochloric acid. However, there was no correlation between chloride and acidity in fifty samples collected during two prolonged periods of high pollution, whereas sulphate (present in concentrations between 40 and 200 mg per g of solids) and acidity were highly correlated. Sulphate was always present in amounts greater than could be accounted for by sulphuric acid alone. These findings suggest that the predominant acid present in air is sulphuric acid.

INTERFERENCE BY PARTICULATE BASES—

Organic bases are produced when coal is distilled, and thus they would be expected to be present in town air. The aliphatic bases and some aromatic bases are readily volatile at ordinary temperatures, but some of the less volatile aromatic bases may be expected to be present in particulate pollution. The latter bases are extremely weak and will not interfere with the titration of sulphuric acid if the pH is carefully chosen. Titration of known mixtures of a 10-fold excess of the aromatic base, aniline, in sulphuric acid, when an extraction solution of low initial pH was used that was titrated back to this pH after the mixture had been added, gave a low result for the acid present; for pH values less than 5·4 a negative amount of acid was indicated. For extraction solutions at pH 7, the true amount of acid was indicated when titrated back to this pH, and therefore such a procedure was chosen for titration of the acid collected from air. For procedures in which solutions of pH >7 were used, spuriously high amounts of acid were indicated. Samples of collected air solids behaved in a similar way to the aniline - sulphuric acid mixture.

Soluble basic particles, such as lime or other metallic oxides or hydroxides, would, if present, interfere with the determination of acid by the titration of filter-paper samples. To find out if such bases are present, the acid in the samples can be neutralised with ammonia gas, and the unaffected bases, if present, determined by titration. This procedure was applied to collected air solids, and the samples were neutralised with ammonia over a period of 2 minutes, after which they were left in the laboratory for 2 hours before being titrated. Samples collected in London were neutralised by exposure to ammonia for 2 minutes and, after being left in laboratory air for 2 hours, were placed in water at pH 7; they were never alkaline but were neutral or slightly acid. This indicated that only negligible amounts of soluble bases were present in the samples. Insoluble bases, such as calcium carbonate, may also be present; their presence can be detected by titrating with sodium tetraborate the "ammoniated" sample after a known excess of acid has been added. Results of these experiments have shown that many samples of solids collected from the air in London contain appreciable amounts of insoluble bases.

In order to overcome the interference by insoluble bases, an amount of 0·01 N sodium tetraborate greater than the equivalent amount of acid (determined first by titrating a portion of the sample at pH 7) is added to 1 to 2 ml of water at pH 7, in which another portion of the sample is immersed; the solution is then titrated with 0·01 N sulphuric acid. This titration procedure allows the sodium tetraborate to neutralise the acid before the insoluble base has a chance to do so. It has been found that the results of the determinations of acid by this method are 10 per cent. higher at times of high pollution and up to 30 per cent. higher at times of low pollution than those obtained by direct titration.

Although the method described above has been found to be suitable for determining acid in town air, two other methods have been considered. In one, the total particulate sulphate is taken to be an indication of the total particulate acid. This "sulphate index" has, however, proved unreliable for use in the City of London, since the acid content has been shown to vary from 20 to 80 per cent. of the amount of sulphate present. In the other method, the acid is neutralised by adding excess of ammonia to the air before the air solids are collected on the filter-paper. The amount of ammonium ion present on the filter-papers was determined with Nessler's reagent 2 hours later, after which time the ammonia not used for neutralisation had come off the filters. The ammonium ion content of an untreated sample of air solids was also measured and the amount of particulate acid calculated from the difference between the two results, it being assumed that all the acid had been converted to ammonium sulphate. This method takes longer than the titration method and the results are not as reproducible, but it has the merit that reactions on the filter-paper between the acid and other material present are minimised.

RESULTS

RANGE OF CONCENTRATION OF PARTICULATE ACID IN TOWN AIR—

The concentrations of particulate acid correspond to those of other pollutants. Concentrations of particulate acid are especially high at times of fog and have reached levels of 678 μg (calculated as sulphuric acid) per cu. metre of air in the City of London. Typical winter daily concentrations are 18 μg per cu. metre of air, compared with 7 μg per cu. metre for summer in the City. The sulphuric acid content of the air in the City of London can be up to 10 per cent. of the total sulphur.

CONCLUSIONS

The procedure developed for the measurement of particulate acid was suitable for its determination in urban air. Interference by other pollutants can be avoided by the use of a back titration technique. The particulate acid appears to be mainly sulphuric acid.

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Iodimetric Determination of Milligram Amounts of Rubber Hydrocarbon

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An iodimetric method is described for determining rubber hydrocarbon; it is based on the bromination of the rubber molecule, and is sensitive to 2 mg of rubber dissolved in benzene. By comparing it with a standard procedure, it has been shown that the proposed method will determine accurately the rubber hydrocarbon content of field latex. The precision with which solutions of pure rubber in benzene can be determined by the method corresponds to a coefficient of variation of about 0·5 per cent., and rubber hydrocarbon in latex can be determined with a precision of about 1·5 per cent.

THE chemical determination of rubber hydrocarbon may be based either on its property of forming a stable tetrabromide, as in early volumetric methods[1,2] and in more recent gravimetric methods,[3,4,5] or on its ability to produce titratable volatile acids on oxidation with chromic acid.[6] Volumetric methods are rapid and sensitive, but, although it has been claimed[7] that rubber hydrocarbon can be satisfactorily determined by bromination, such procedures have not been found generally satisfactory. Their unsatisfactory nature may have been caused by faulty analytical techniques, and in the work described here an improved analytical procedure has been used for determining rubber hydrocarbon, after bromination of the rubber molecule under carefully controlled conditions.

Bromination methods assume that the rubber hydrocarbon tetrabromide is formed by the addition of bromine at double bonds, as summarised in the equation—

$$(C_{10}H_{16})_n + 2n\text{Br}_2 = (C_{10}H_{16}\text{Br}_4)_n$$

Attempts have been made to allow for any extra bromine taken up through substitution by applying the McIlhiney correction,[1,2] but according to Bloomfield[8] the simultaneous absorption of halogen by cyclisation makes this impracticable. Substitution can, however, be suppressed by using a polar reagent; for example, bromine in glacial acetic acid, as in the work of Kemp and Mueller.[9]

For volumetric methods involving determination of the excess of bromine remaining after bromination, it is essential that a quantitative addition of the halogen should be made initially. Preliminary work had shown that such addition is extremely difficult when, as in the methods referred to above,[1,2] solutions of bromine in a volatile solvent, such as carbon tetrachloride, are used. Solutions of this kind quickly lose bromine on exposure to the air; this is also true for solutions of bromine in acetic acid. Quantitative introduction of bromine can be readily made, however, by using bromide - bromate mixtures,[10] and a polar reagent of this type has been used in the method of determining rubber hydrocarbon described below.

The proposed method is designed for rubber that is completely soluble in benzene, since Willits, Swain and Ogg[3] have shown by a gravimetric procedure that such a method can be applied to the determination of rubber in plant tissue. This application, which involves problems of extraction and of interference by other substances, is not discussed in this paper, but it is intended to show in a later publication how the proposed method can be used for determining rubber in plants.

EXPERIMENTAL

Before its determination rubber may be dissolved in carbon tetrachloride,[1,2] chloroform[7] or benzene.[3] Benzene has been found most suitable for extracting rubber from plant tissue[11] and, since one object of this paper is to describe a method also applicable to the analysis of plant material, the use of solvents other than benzene has not been studied.

BROMINATION OF SOLUTIONS OF RUBBER IN BENZENE—

Preliminary work had shown that both commercial and analytical-reagent grades of benzene absorbed appreciable amounts of bromine; further, when the same sample of benzene was repeatedly brominated, the amount of bromine absorbed each time rapidly decreased with successive brominations. Purification of benzene by distillation alone did not reduce the amount of bromine absorbed, but washing with concentrated sulphuric acid produced a large decrease. These results suggested that much of the bromine was absorbed by an impurity such as thiophen, which would be removed by sulphuric acid but not easily by distillation, as it boils at about the same temperature as benzene.

The amount of bromine absorbed by rubber in solution must be obtained by subtracting the amount absorbed by the solvent from the total amount taken up by the solution, and for accurate work, therefore, purified benzene should be used. In Fig. 1 values obtained by subtraction in this way show the rate at which bromine is absorbed by rubber dissolved in purified benzene; the corresponding absorption of bromine by the solvent is also shown.

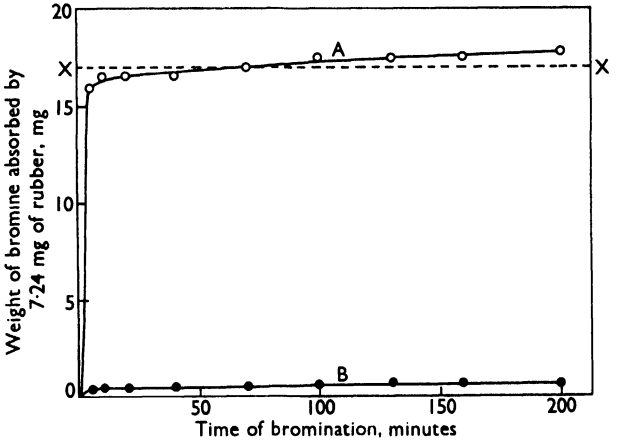

Fig. 1. Effect of time on the bromination of benzene and rubber hydrocarbon: curve A, purified rubber; curve B, purified benzene

The effect of external conditions on the absorption of bromine was also investigated; rubber dissolved in purified benzene was brominated for 100 minutes, in clear bottles in both subdued and bright light and also in opaque bottles in a dark cupboard at various temperatures between 6° and 28° C. The results are shown in Table I, and indicate that the effect of light on the bromination of benzene is sufficiently pronounced to make it essential that, in the proposed procedure, bromination is carried out in the dark. The results also show that the effect of temperature on the bromination of both benzene and rubber in the dark is small compared with its effect in subdued or bright light.

TABLE I

EFFECT OF LIGHT AND TEMPERATURE ON THE BROMINATION OF BENZENE AND RUBBER

The results are expressed as milligrams of bromine absorbed by 10 ml of purified benzene and by 8·92 mg of purified rubber

	Bromine absorbed					
	In the dark by—		In subdued light by—		In bright light by—	
Temperature, °C	benzene	rubber	benzene	rubber	benzene	rubber
7	0·32	21·5	—	—	—	—
20	0·54	22·4	17·6	23·9	—	—
27	0·74	23·9	32·8	14·1	72·6	1·1

METHOD

REAGENTS—

The reagents should be prepared from pure chemicals and distilled water.

Sodium molybdate solution, 0·1 *per cent. w/v in* 10 *per cent. v/v hydrochloric acid and* 10 *per cent. v/v acetic acid.*

Potassium bromate solution, 0·55 *per cent. w/v in* 10 *per cent. w/v potassium bromide solution.*

Sodium borate solution, 2·5 *per cent. w/v, aqueous.*

Sodium thiosulphate solution, 2·5 *per cent. w/v, aqueous.*

Sodium thiosulphate solution, 0·04 N, *aqueous.*

Iodine solution, 0·01 N *in* 4 *per cent. w/v potassium iodide solution.*

Benzene—Purify by shaking with concentrated sulphuric acid and washing four times with water. Filter the benzene, and then distil at 80° C; discard the first and last 50 ml of distillate from 2 litres.

PROCEDURE—

Put a 25-ml portion of a benzene solution containing 5 to 10 mg of rubber hydrocarbon into an opaque stoppered bottle, and add 5 ml of sodium molybdate solution and then 5 ml exactly of potassium bromate solution. Replace the stopper tightly, mix the aqueous and benzene phases thoroughly, and place the bottle in a dark cupboard. After 100 minutes have elapsed record the temperature of the cupboard, remove the bottle, and quickly add 2 g of potassium iodide to the contents. Replace the stopper tightly, shake the solution thoroughly, and after 5 minutes quickly add 100 ml of sodium borate solution. (A 100-ml calibrated flask with a spout permits rapid addition of sodium borate to be made; it is essential that this flask and all glass apparatus used in the bromination should be free from grease.) Again shake the solution thoroughly, and add 25 ml of sodium thiosulphate solution with continuous swirling. After 5 minutes, filter about 20 ml of the solution through a Whatman No. 1 filter-paper (previously moistened with distilled water to ensure that only the aqueous phase passes through), and discard. Filter the remainder of the aqueous phase into a clean dry flask, and titrate a 50-ml portion with the standard iodine solution and starch as indicator. Carry out a blank determination on 25 ml of benzene only by the same procedure. The volume of standard iodine solution corresponding to the bromine absorbed by the hydrocarbon is obtained by multiplying the difference between the two titres by the total volume of the aqueous phase (135 ml) and dividing by the volume of the portion taken (50 ml). The amount of rubber present can then be calculated by multiplying this result by a factor, corrected for temperature as described below.

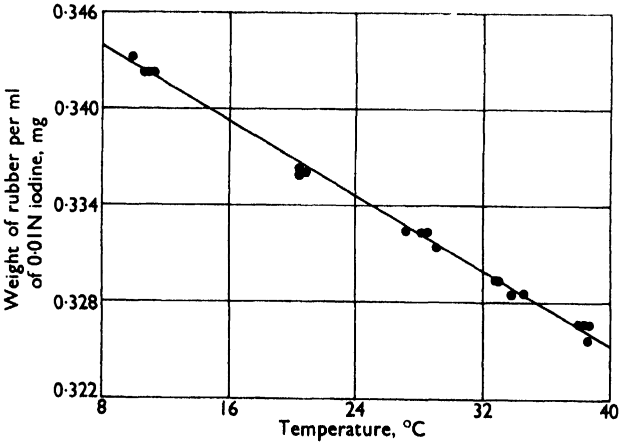

Fig. 2. Variation with temperature of an empirical factor for calculating rubber hydrocarbon

CALIBRATION OF THE METHOD—

Fig. 1 indicates that there is a rapid initial absorption of bromine by rubber hydrocarbon, caused mainly by addition of the halogen at double bonds; subsequently there is a much slower absorption that may be caused partly by substitution of hydrogen. If this is so, bromination will not always produce the tetrabromide quantitatively, but it can be experimentally shown with considerable precision that at a given temperature the amount of bromine absorbed per milligram of rubber is nearly constant over the range 2·5 to 12·5 mg; an empirical factor can therefore be used to relate the weight of rubber to the amount of bromine absorbed. It was clear from the results shown in Table I that the factor would vary with temperature, and the nature of this variation is shown in Fig. 2; these results were obtained by brominating in accordance with the proposed procedure 8 mg of purified rubber in 25 ml of purified benzene. A highly significant linear regression of an empirical factor (expressed as mg of rubber per ml of 0·01 N iodine) on temperature is shown, with a negative slope corresponding to the equation—

$$\text{Factor at } t^\circ \text{ C} = 0{\cdot}3339 - 0{\cdot}000589\,(t - 25).$$

A similar calibration, carried out with synthetic *cis*-polyisoprene as the standard substance, gave a slightly different factor with a more pronounced temperature gradient corresponding to the equation—

$$\text{Factor at } t^\circ \text{ C} = 0{\cdot}3345 - 0{\cdot}00126\,(t - 25).$$

COMPARISON WITH A STANDARD METHOD FOR ANALYSING LATEX

The dry rubber content of field latex, which includes rubber hydrocarbon together with small amounts of other materials,[12] was determined by a standard method,[13] and rubber hydrocarbon was determined by the proposed method, after non-rubber substances capable of absorbing bromine had been removed by means of the pre-treatment described below.

Weigh 0·15 to 0·20 g of latex into a 250-ml beaker, and spread by adding 1 ml of water and rotating. Evaporate to dryness on a steam-bath at 90° C, when an extremely thin translucent film should form on the bottom of the beaker. Extract three times with 100 ml of boiling ethanol, 15 minutes being taken for each extraction. After the final extract has been removed by decantation, heat on the steam-bath until free from ethanol. Add 150 ml of purified benzene, stir thoroughly at intervals, and, when all the rubber has dissolved, make up to 250 ml in a calibrated flask. Determine the content of rubber hydrocarbon in a 25-ml portion by the procedure described above.

The results of the comparison were plotted (see Fig. 3) and reveal a highly significant regression of rubber hydrocarbon on dry rubber content.

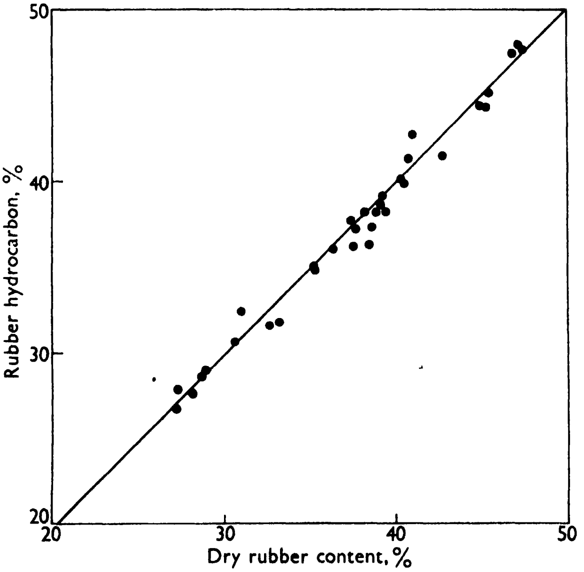

Fig. 3. Regression of rubber hydrocarbon in field latex upon dry rubber content

DISCUSSION OF THE METHOD

The amount of bromine absorbed, by both hydrocarbon and solvent, is equal to the difference between the amount added and the amount remaining at the end of the reaction; an excess of potassium iodide converts the latter into an equivalent amount of iodine, which can then be titrated with thiosulphate.

The presence of three phases (aqueous, benzene and rubber bromide) had made the direct titration of liberated iodine difficult, the more so when bromination was carried out in opaque bottles. Moreover, as pointed out by Fisher, Gray and Merling,[2] traces of excess of bromine, occluded in the precipitated bromide, may not be released during the titration. These difficulties are avoided in the proposed method by adding a measured excess of thiosulphate and by waiting until its reaction with the halogen is complete; the aqueous phase is then filtered off, a portion of it is taken and the excess of thiosulphate is titrated with standard iodine solution. Thiosulphate is however unstable in contact with the amount of acid needed to release bromine from bromide - bromate mixtures, and for accurate titrations a definite pH is needed, depending on the concentrations of iodine and thiosulphate used.[10] In the proposed procedure the acid is partly neutralised with sodium borate before the addition of thiosulphate, and a borate - acetate buffer giving a final pH of 5·5 is thus formed.

Another difficulty had arisen when a strong acid only was used to liberate bromine from bromide - bromate mixtures; this acid (hydrochloric) produced an excessive amount of heat when neutralised by sodium borate. The problem was eventually solved by replacing part of the hydrochloric acid by acetic acid, which liberates less heat on neutralisation, and by adding sodium molybdate, which strongly catalyses the release of bromine under such conditions.[10]

In Fig. 1, the line × - × marks an absorption of 17 mg of bromine by 7·24 mg of rubber; this amount corresponds to a quantitative formation of rubber hydrocarbon tetrabromide, which contains 70·13 per cent. of bromine, and the minimum bromination time required to form it appears to be about 70 minutes. The rate of absorption of bromine seems, however, to fall off appreciably after 100 minutes, and since this time has been used by other workers[5] it has been adopted as the bromination time in the proposed procedure.

ACCURACY AND PRECISION OF THE METHOD—

The calibrations recorded above show that similar results are obtained when purified rubber and *cis*-polyisoprene are used, although the effect of temperature on bromination is not exactly the same for both substances. It is, however, accepted that there are definite differences in structure between *cis*-polyisoprene and natural rubber.[14] Moreover, recent work[15] has suggested the presence of aldehyde and other abnormal groups in the natural rubber molecule; both these variables might be expected to cause a difference in bromination between the two materials, when the procedure described above is employed. The *cis*-polyisoprene is considered to have a rubber hydrocarbon content of between 99·5 and 100 per cent.; the purified natural rubber, from which inorganic matter, proteins and fatty materials had been removed by treatment with an aqueous detergent and alcohol, should be of similar purity. The fact that calibrations made by using the two standards have given similar results can therefore be taken as an indication of the satisfactory accuracy of the method.

The accuracy of the method can also be judged by comparing it with a standard procedure[13] for determining the dry rubber content of field latex. The results of the comparison are recorded in Fig. 3, in which mean rubber hydrocarbon values (obtained from duplicate brominations on each of two sub-samples of latex) are plotted against the means of two determinations of dry rubber content on the same samples of latex.

The linear correlation between rubber hydrocarbon (R.H.C.) and dry rubber content (D.R.C.) is highly significant (correlation coefficient + 0·989), and the corresponding regression equation is—

$$\text{R.H.C.} = \text{D.R.C.} \times \underset{\substack{\text{(standard error}\\ \pm\ 0{\cdot}0268)}}{1{\cdot}0134} - \underset{\substack{\text{(standard error}\\ \pm\ 1{\cdot}013)}}{0{\cdot}580}$$

The deviations of the slope (1·0134) and the intercept (—0·580) from unity and zero, respectively, are not statistically significant, and the least squares regression differs only insignificantly from the relation R.H.C. = D.R.C.; this can be readily shown in terms of the total variation among the 34 samples—the least squares regression accounts for 97·81 per cent. of the variation, whereas the alternative relation (R.H.C. = D.R.C.) accounts for 97·78 per cent. This implies, but does not establish, that in this comparison of methods R.H.C. is equivalent to D.R.C.; but it should be noted that the regression equation given above has been worked out for field latex only—when other lattices are involved, for example, skim or concentrate, it may be necessary to re-determine the equation, but the same method would seem to be applicable.

Table II summarises the statistical examination of the rubber hydrocarbon results obtained in the comparison of methods. The components of variance show that considerably more error is associated with sub-sampling and pre-treatment (which are confounded) than with bromination and titration (which are also confounded). By combining components for these sources of variation, the standard error of a single determination of rubber hydrocarbon, on any given sample of latex, can be estimated as ±0·5712, which corresponds to a coefficient of variation of 1·53 per cent. The coefficient of variation associated with duplicate brominations is, however, 0·48 per cent. and this figure agrees well with a value of 0·35 per cent. computed for duplicate determinations, the means of which are plotted in Fig. 2.

TABLE II

STATISTICAL ANALYSIS OF RUBBER HYDROCARBON DETERMINATIONS ON 34 SAMPLES OF LATEX

Source of variation	Degrees of freedom	Mean square	Component of variance
Between samples of latex	33	140·7858***	σ_2^2 = 35·0412
Between sub-samples within samples of latex	34	0·6210***	σ_1^2 = 0·2948
Between duplicate brominations on sub-samples of latex	68	0·0315	σ_0^2 = 0·0315
Total	135	—	—

Mean rubber hydrocarbon content = 37·296 per cent. by weight.

Standard error of a single determination on a given sub-sample = $\pm\sigma_0$ = + 0·1775; coefficient of variation = 0·48 per cent.

Standard error of a single determination on a given sample = $+\sqrt{\sigma_0^2 + \sigma_1^2}$ = ± 0·5712; coefficient of variation = 1·53 per cent.

*** Significant at the 0·1 per cent. level.

CONCLUSIONS

The proposed method shows that milligram amounts of rubber hydrocarbon soluble in benzene can be rapidly determined by iodimetric titrations, after preliminary bromination of the rubber molecule. Various experimental conditions necessary to attain satisfactory accuracy and precision are specified. They include exclusion of light during a definite bromination period, correction for different brominating temperatures, the use of an inorganic brominating reagent in order to ensure quantitative addition of bromine, and careful control of the procedure used for the iodimetric determination. The determination of rubber hydrocarbon by the proposed method is also shown to be closely correlated with the determination, by a standard method, of the dry rubber content of field latex.

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Determination of Arsenic in Copper and Copper-base Alloys

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Existing procedures for determining arsenic in copper and its alloys have known limitations and disadvantages, especially when less than about 100 p.p.m. of arsenic are present and a 5-g sample is not available.

A procedure has been developed, involving not more than 1 g of sample, and successfully applied to the analysis of typical samples, including copper-base alloys containing up to 0·2 per cent. of arsenic. Limited tests have shown the method to have a potential application in ferrous analysis.

The sample is dissolved in a hydrochloric acid - hydrogen peroxide mixture; quinquivalent arsenic is reduced with hypophosphorous acid, and an empirical extraction of the arsenious chloride is made into chloroform. The recovered arsenic is oxidised to the quinquivalent state and then determined absorptiometrically after reduction of arsenomolybdate to molybdenum blue.

The recommended procedure is suitable for determining arsenic down to at least 5 p.p.m. in copper and 20 p.p.m. in brass, bronze or cupro-nickel; it is not subject to interference from alloying elements and impurities normally present in these materials. A single determination takes about 2 hours, but at least 8 determinations can be completed by one analyst in a normal working day.

SEVERAL methods are available for determining arsenic in non-ferrous materials, but none is entirely suitable for amounts of less than 100 p.p.m. when the sample weight is restricted to 1 g or less. Although such amounts of arsenic would normally be determined spectrographically, there is still a need for an accurate and sensitive method for analysing standard samples.

Two procedures are given in B.S. 1800,[1] one based on a preliminary distillation of arsenious chloride, the other on an initial precipitation, with hypophosphorous acid, of elemental arsenic, together with selenium and tellurium, and then by separation of interfering elements; both procedures are completed by titration with a standard iodine solution. These two methods have known limitations and disadvantages; *e.g.*, in both procedures, when the amount of arsenic present is small, the volume of standard iodine solution is also small, and a minimum sample weight of 5 g is therefore necessary when the arsenic content is less than about 100 p.p.m.

Other methods are available for determining arsenic in non-ferrous materials,[2,3,4,5,6] but phosphorus, selenium, tellurium and silicon, which are present in some grades of copper and its alloys, interfere in one or other of these methods.

The separation of arsenic by precipitation with hypophosphorous acid is only 90 to 95 per cent. complete[7]; selenium and tellurium are also precipitated. This separation has been used by Case[8] in the preliminary stages of a method for determining arsenic at levels above 0·1 mg in copper-base alloys before determining the arsenic as molybdenum blue. Experiments in these laboratories have shown that, when the amount of arsenic separated in this way is less than 0·1 mg, the efficiency of separation is variable—frequently less than 90 per cent.—and that selenium suppresses development of the molybdenum-blue colour. Variable recoveries of arsenic after precipitation with hypophosphorous acid seem to be associated with conditions for precipitation and subsequent washing of the elemental arsenic. Opinions on optimum conditions for these operations are conflicting, and the authors' work has failed to improve the reproducibility of the procedure for determining arsenic below 100 p.p.m.

Tervalent arsenic can be extracted from a hydrochloric acid solution into certain organic solvents; *e.g.*, from 11 M hydrochloric acid, recoveries of 94 per cent. with benzene[9] and about 75 per cent. with carbon tetrachloride[10] have been reported. These observations were made the basis of subsequent experimental work designed to provide a satisfactory method for determining small amounts of arsenic when the weight of sample was restricted to about 1 g. It was proposed to complete the determination absorptiometrically by the method described by Case.[8]

EXPERIMENTAL

ABSORPTIOMETRIC DETERMINATION OF ARSENIC—

A calibration graph was prepared, without involving an extraction, by using the colour development conditions recommended by Case.[8]

A standard solution of tervalent arsenic was prepared by dissolving arsenious oxide in sodium hydroxide solution. Volumes of this solution, containing from 0·01 to 0·1 mg of arsenic, were placed in separate 50-ml calibrated flasks. To the contents of each flask were added 5 drops of 0·1 N iodine, to oxidise tervalent arsenic, 5·0 ml of a 1 per cent. w/v solution of ammonium molybdate in dilute sulphuric acid (1 + 6) and 2·0 ml of 0·15 per cent. w/v hydrazine sulphate solution. Each flask was immersed in a boiling-water bath for 10 minutes, cooled, and diluted to the mark; the optical densities were then measured at 8400 Å in 2-cm cells.

The relation between optical density and concentration of arsenic was linear, 0·05 mg of arsenic corresponding to an optical density of 0·69.

FORMATION AND EXTRACTION OF TERVALENT ARSENIC—

Tests in which tervalent arsenic was extracted from 11 M hydrochloric acid into benzene, chloroform or carbon tetrachloride showed that the efficiency of extraction decreased in this order and, although benzene was over 20 per cent. more efficient than chloroform, separation of the organic phase was less defined than when chloroform was used. From 50 ml of 11 M hydrochloric acid containing 0·05 mg of tervalent arsenic, 71 per cent. of the arsenic was extracted by 25 ml of chloroform, and this amount decreased with decrease in the concentration of acid.

Based on these observations, conditions for extracting arsenious chloride were standardised, and a series of standards was prepared and examined. Volumes of a standard solution of tervalent arsenic, containing 0·01 to 0·1 mg of arsenic were placed in separate 100-ml separating funnels, each containing 50 ml of concentrated hydrochloric acid. To the contents of each funnel were then added 25 ml of chloroform, the mixture was shaken vigorously for 3 minutes, and the aqueous layer was discarded. The chloroform was then shaken for 1 minute with 20 ml of water, and discarded. The aqueous layer was run into a 50-ml calibrated flask, 5 drops of 0·1 N iodine were added, and the molybdenum-blue complex was developed as described earlier. Recoveries were plotted, and the resulting graph was linear (0·05 mg of arsenic corresponding to an optical density of 0·48), indicating that extraction of arsenious chloride into chloroform could provide a suitable method for determining arsenic.

Because the sample must be dissolved under oxidising conditions, to prevent loss of arsenic, arsenic is maintained in the quinquivalent state during solution of the sample, and, as such, less than 5 per cent. is extracted into chloroform. Attempts to reduce quinquivalent arsenic to the tervalent state in solutions of cupric chloride in concentrated hydrochloric acid with hydroxyammonium chloride or hydrazine hydrochloride were unsuccessful; both reagents were unsuitable, and subsequent recoveries of arsenic were low. Improved, although variable, recoveries were obtained when stannous chloride was used as a reducing agent.

At temperatures below about 50° C, quinquivalent arsenic in 6 M hydrochloric acid is reduced by hypophosphorous acid[11] to tervalent arsenic, and not, as might be expected, to elemental arsenic. It was also found that, when quinquivalent arsenic was reduced with hypophosphorous acid, provided copper was completely reduced to the cuprous state, nearly 70 per cent. of the arsenic present in a cupric chloride - 11 M hydrochloric acid solution was recovered by a single extraction into 25 ml of chloroform at 20° C.

When this reduction procedure was applied to a sample of vacuum-cast copper, arsenic values, obtained by the molybdenum-blue method, were high and variable, but if the chloroform extract was washed with 11 M hydrochloric acid before the arsenious chloride was extracted, which presumably had the effect of removing entrained phosphorus-containing salts, the values were low (as expected) and consistent.

A graph obtained under these conditions was linear, although of lower slope than the earlier graphs, 0·05 mg of arsenic corresponding to an optical density of 0·44. The values obtained agreed with the values calculated from the known distribution of tervalent arsenic between chloroform and hydrochloric acid, indicating that complete reduction of tervalent arsenic had been achieved. It was also found that equilibrium between the chloroform and the 11 M acid phase was established within 1 minute of shaking.

To extend the range of application of the procedure to determining arsenic in excess of 100 p.p.m., the effect of extracting 0·06 mg of arsenic from solutions containing from 0·1 to 1 g of copper was examined. Results showed that the amount of tervalent arsenic extracted increased progressively with increase in copper concentration, but became constant when 0·4 g or more of copper was present (see Table I). This observation was not altogether surprising, because it is known that a relatively large amount of copper must be present in order to achieve quantitative reduction of quinquivalent arsenic with hypophosphorous acid. To make the calibration graph independent of sample weight, therefore, the amount of copper present should be greater than 0·4 g, and, in subsequent experiments, pure copper was added to the weighed sample, when necessary, so that the total weight of copper present was 1 g.

TABLE I

EFFECT OF COPPER ON EXTRACTION OF 60 p.p.m. OF ARSENIC

Copper present, g	Optical density (2-cm cells; 8400 Å)	Copper present, g	Optical density (2-cm cells; 8400 Å)
0·1	0·445	0·5	0·556
0·2	0·480	0·7	0·558
0·3	0·540	0·8	0·558
0·4	0·558	1·0	0·562

EFFECT OF REAGENTS—

The extraction of tervalent arsenic into chloroform is governed by a distribution law, and therefore the effect of altering the amounts of the various reagents had to be considered. The amounts of hydrochloric acid and chloroform for the first extraction were fixed at 60 and 25 ml, respectively; these volumes were convenient for manipulation purposes. A 1-ml variation in the volume of the hydrochloric acid did not significantly affect the recovery of arsenic, and this reagent was added from a measuring cylinder. A similar variation in volume of chloroform was significant, and addition was made from a pipette. Salts entrained in the chloroform extract were reduced to an insignificant level by washing with 10 ml of concentrated hydrochloric acid, and, although the volume of water necessary to remove arsenic from the chloroform extract could be as low as 5 ml, 20 ml was used to dilute any residual hydrochloric acid in the separating funnel, and also to allow the aqueous extract to be conveniently washed into a 50-ml calibrated flask. The hydrochloric acid and water were both added from measuring cylinders.

TABLE II

RECOVERY OF ARSENIC FROM HIGH-CONDUCTIVITY COPPER

Sample No.	Element added	Amount of element added, %	Amount of arsenic added, p.p.m.	Amount of arsenic found, p.p.m.
V.C. A	Germanium	0·1	60	58
	Selenium	0·1	60	57
	Tellurium	1	60	61
V.C. A	—	—	50	50·7, 49·3, 49·6, 49·8, 50·5, 50·6, 51·2, 50·1
C.C.	—	—	Nil	4
			5	7
L.V. 6	—	—	Nil	4
			5	8
W.P.	—	—	Nil	7
			5	14
B. 19	—	—	Nil	60
			5	65

The concentration of hypophosphorous acid was shown to be unimportant, provided it was sufficient to reduce copper completely to the cuprous state. However, to maintain a constant acidity the volume of this reagent added was fixed at 3·0 ml.

Provided the acid ammonium molybdate and hydrazine sulphate solutions were freshly prepared, the calibration graph was reproducible. Because the molybdenum-blue reaction is dependent on pH, these reagents were added from a pipette.

EFFECT OF OTHER ELEMENTS—

Samples of vacuum-cast copper (1 g), to which had been added a standard solution equivalent to 60 p.p.m. of arsenic and solutions of germanium, selenium or tellurium, were examined by the recommended procedure. No interference was observed from the presence of 0·1 per cent. of either germanium or selenium. Selenium and tellurium were both precipitated by hypophosphorous acid, but, whereas selenium accumulated on top of the chloroform interface and remained in the separating funnel when the chloroform was removed, the presence of 1 per cent. of tellurium obscured the phase boundary and made a clear separation of the phases difficult. This difficulty was overcome by filtering the reduced solution through a Whatman No. 542 filter-paper before extraction into chloroform (see Table II).

The information contained in Table III indicates that the alloying constituents and impurities present in these samples do not interfere in the proposed procedure.

TABLE III

ANALYSIS OF MISCELLANEOUS SAMPLES

Sample		Arsenic found by— proposed procedure, p.p.m.	alternative procedure, p.p.m.	Certificate value, p.p.m.
Brass (70/30)	No.	36, 36	39	—
	No. 11	21, 23	17	—
	No. 15	35, 35	33	—
	No. 17	1860, 1870	1930	—
Brass (85/15)	No. 8	48, 46	45	—
	No. 9	71, 70	70	—
	No. 10	53, 53	49	—
	No. 21	30, 32	31	—
	No. 22	130, 129	140	—
Everdur S185 (96 Cu/3 Si/1 Mn)		100, 100	90	—
Arsenic de-oxidised copper, S186		3400, 3400	3500	—
Chrome copper S187 (99 Cu/0·5 Cr/0·3 Zn/0·2 Ni)		59, 62	63	—
Alumbro M5363 (78 Cu/20 Zn/2 Al)		410	420	—
B.C.S. 183 Bronze A (85 Cu/10 Sn/2 Zn/2 Pb/0·25 P)		620	—	500 to 600*
B.C.S. 179 Manganese brass B (59 Cu/24 Zn/1 Mn/1 Fe/2 Sn/1·5 Al/2 Pb/1 Ni)		190	—	200 to 400*
B.C.S. 207 Bronze C (86 Cu/10 Sn/2·5 Zn/0·1 Ni/0·5 Pb)		620	—	500
B.C.S. 183/1 Bronze (85 Cu/5 Sn/5 Zn/4 Pb/0·5 P/0·5 Ni)		1380	—	1400
B.C.S. 180/1 Cupro-nickel (67 Cu/31 Ni/0·8 Fe/0·8 Mn)		120, 119, 118	—	50 to 80*
B.C.S. 239/1 Carbon steel		370, 360	—	330

* These certificate values are not standardised. Comparisons are given to indicate that the values obtained by the proposed procedure are likely to be correct and that the procedure is applicable to these materials.

APPLICATION OF THE PROCEDURE—

Several samples of brass (85 per cent. of copper, 15 per cent. of zinc and 70 per cent. of copper, 30 per cent. of zinc) were examined by the proposed procedure. They were also analysed by a method in which arsenic was determined absorptiometrically as described in the proposed procedure, but after a preliminary distillation of arsenic; good agreement was obtained in all these tests (see Table III).

The precision of the proposed procedure was established by examining a 10-g sample of pure copper to which had been added, as a standard solution of arsenic, the equivalent of 50 p.p.m. of arsenic. The sample was dissolved in a hydrochloric acid - hydrogen peroxide mixture, and arsenic was determined in eight aliquots each containing the equivalent of 1 g of copper. The standard deviation obtained was 0·6 p.p.m., and the maximum deviation was 0·9 p.p.m. (see Table II).

The procedure was also applied to British Chemical Standard samples of leaded tin-bronze, manganese-brass and cupro-nickel, and the arsenic values found were in agreement with those reported in the Certificates; most of the arsenic values are quoted in the Certificates over a relatively wide range. When the method was applied to copper-base alloys containing alloying amounts of silicon, aluminium or chromium, the arsenic values found were in good agreement with those obtained by alternative procedures. In the examination of chromium-bearing samples it was necessary to dissolve the sample with the aid of perchloric acid, to ensure complete solution of chromium.

Limited tests on a sample of carbon steel to which 1 g of copper had been added indicated that the procedure could be extended to ferrous materials.

These values are summarised in Table III.

METHOD

REAGENTS—

Standard arsenic solution—Dissolve 0·132 g of arsenious oxide, previously dried at 105° C, in 5 ml of warm 5 per cent. w/v sodium hydroxide solution. Dilute to 100 ml, add diluted sulphuric acid (1 + 1) until the solution is just acid to litmus paper, and then dilute to 1 litre. Dilute 50 ml of this solution to 250 ml.

1 ml ≡ 0·02 mg of arsenic.

Ammonium molybdate solution, 1 per cent. w/v—Add, slowly, 14 ml of concentrated sulphuric acid to 60 ml of water, and then dissolve 1 g of ammonium molybdate in the warm solution. Cool, and dilute to 100 ml.

This reagent must be freshly prepared.

PREPARATION OF CALIBRATION GRAPH—

Place, separately, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of the standard arsenic solution (1 ml ≡ 0·02 mg of arsenic) in each of five 100-ml beakers containing 1·0 g of pure copper; use a sixth beaker containing 1·0 g of copper for a blank determination.

Continue with each beaker as described below.

Add 5 ml of concentrated hydrochloric acid, and then place in a cold-water bath. Add 3 ml of 100-volume hydrogen peroxide, allow the initial reaction to subside, and then add a further 7 ml of the hydrogen peroxide. When solution of the sample is complete, remove the beaker from the water bath, and allow the solution to simmer at the side of a hot-plate to decompose the excess of hydrogen peroxide; finally, evaporate the solution to dryness.

To the residue add 50 ml of concentrated hydrochloric acid, stir to dissolve soluble salts, adjust the temperature to 20° C, add 3·0 ml of 50 per cent. w/w hypophosphorous acid, and then set the solution aside for 5 minutes. Transfer the solution to a dry separating funnel, with the aid of 10 ml of concentrated hydrochloric acid, add 25·0 ml of chloroform, and then shake vigorously for 1 minute. Allow the two layers to separate, and run the lower (chloroform) layer into a dry separating funnel; discard the aqueous layer. Shake the chloroform extracts with 10 ml of concentrated hydrochloric acid for 30 seconds. Allow the two layers to separate, and run the lower (chloroform) layer into a dry separating funnel; discard the aqueous layer. Add 20 ml of water, and shake vigorously for 1 minute; allow the two layers to separate, discard the lower (chloroform) layer, and transfer the aqueous layer to a dry 50-ml calibrated flask with about 5 ml of water.

Add the reagents listed below in the order stated; wash down the neck of the flask, and mix well after each addition—

5 drops of 0·1 N iodine solution;
5·0 ml of 1 per cent. w/v ammonium molybdate solution;
2·0 ml of freshly prepared 0·15 per cent. w/v hydrazine sulphate solution.

Stand the flask in a boiling-water bath for 10 minutes, then cool to 20° C, and dilute to the mark.

Measure the optical density at 8400 Å in 2-cm cells.

PROCEDURE—

Dissolve 1 g of the sample (see Notes 1 and 2), and continue as described under "Preparation of Calibration Graph" (see Note 3).

With each batch of samples, simultaneously determine a reagent blank value on 1 g of pure copper.

NOTES—

1. Applicable to samples containing up to 100 p.p.m. of arsenic. For arsenic contents above this range, use a proportionally smaller weight of sample, and add sufficient pure copper to bring the weight of copper to 1 g.

2. In the examination of alloys containing chromium, *e.g.*, Kumium, dissolve the sample in 5 ml of perchloric acid, sp.gr. 1·54, and 1 ml of concentrated nitric acid, and then evaporate to dryness. Dissolve the residue in 50 ml of concentrated hydrochloric acid, and continue as described under "Preparation of Calibration Graph."

3. In the examination of alloys containing tellurium, above about 0·1 per cent., redissolve the residue, after evaporation, in 30 ml of concentrated hydrochloric acid. Adjust the temperature to 20° C, add 3·0 ml of the hypophosphorous acid, and set aside for 5 minutes. Filter the solution through a Whatman No. 542 filter-paper into a dry separating funnel, and wash the filter-paper with three 10-ml portions of concentrated hydrochloric acid. Continue as described under "Preparation of Calibration Graph."

CONCLUSIONS

The proposed procedure is suitable for determining arsenic down to at least 5 p.p.m. in copper and 20 p.p.m. in copper-base alloys containing common alloying constituents. Selenium and tellurium up to at least 0·1 and 1 per cent., respectively, do not interfere. A single determination takes about 2 hours, but at least 8 determinations can be completed by one analyst in a normal working day; the precision of the method is within 1 p.p.m. at the 50 p.p.m. of arsenic level.

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The Determination of Arsenic in Germanium Dioxide

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A method is described in which tervalent arsenic is separated from germanium in oxalic acid solution by extraction with diethylammonium diethyldithiocarbamate in chloroform. The extracts are scrubbed with dilute oxalic acid, evaporated successively with nitric - perchloric acid, hydrochloric acid and, finally, evaporated to fumes with sulphuric acid. After addition of zinc to the diluted sulphuric acid, arsine is absorbed in a pyridine solution of silver diethyldithiocarbamate to form a red compound, the optical density of which is measured spectrophotometrically. A radioisotope of arsenic was used to find the best conditions for extraction. Variables in the evolution procedure are discussed.

The method can be used for determining down to 0·1 µg of arsenic with an error of ±0·05 µg; this corresponds to 0·02 p.p.m. of arsenic in the germanium dioxide sample.

ARSENIC is an extremely undesirable impurity in germanium used in the manufacture of transistors. It is consequently necessary to examine samples of germanium dioxide, which is used as a raw material, to determine their arsenic content. The maximum acceptable limit for arsenic in germanium dioxide is 0·1 p.p.m.; hence a suitable method of analysis is one that can determine arsenic within the range 0·02 to 0·1 p.p.m.

The literature was studied, and the methods of Payne[1] and Luke and Campbell[2] were used as starting points for the subsequent work. Payne dissolved the sample in a mixture of oxalic acid and ammonium oxalate, reduced the arsenic to the tervalent state, and extracted it with diethylammonium diethyldithiocarbamate in chloroform. After wet oxidation of the extract and heating until fumes of sulphuric acid were evolved, the solution was diluted and the arsenic evolved electrolytically as arsine. This was passed over a cotton thread impregnated with mercuric chloride, and the length of the black stain produced after treatment with silver nitrate was a measure of the arsenic present. Similar methods of dissolution and separation were used by Luke and Campbell, but, after wet oxidation of the organic complex in the chloroform layer, evaporation with hydrochloric acid was carried out to remove any entrained germanium. The solution was then heated until fumes of sulphuric acid were evolved, diluted, and the arsenic determined by a molybdenum-blue procedure.

Little modification was needed to the dissolution and extraction steps in these methods, but the electrolytic Gutzeit determination of the traces of arsenic occasionally gave non-reproducible stains and also involved setting the solution aside overnight. The final molybdenum-blue procedure was subject to interferences, presumably from silica derived from the Pyrex glassware. The blank values of the acid, ammonium molybdate and hydrazine sulphate were equivalent to between 0·5 and 0·7 µg of arsenic, and the difference between replicates as well as the blank values were considered unsatisfactory for the determination of traces of arsenic. For determining more than a few micrograms up to several hundred micrograms of arsenic, however, the molybdenum-blue method is excellent.

Silver diethyldithiocarbamate was first described as a reagent for arsenic by Vašák and Šedivec[3]; it has since been used in the analysis of iron products,[4] naphthas,[5,6] petroleum products and catalysts.[7] The method involves evolution of arsine and its absorption in a solution of the reagent in pyridine. Work in this laboratory has shown that microgram amounts of germanium interfere with this procedure, so that quantitative separation of germanium from the arsenic is essential.

EXPERIMENTAL

SEPARATION—

Five grams of germanium dioxide and 18 g of oxalic acid were weighed into a 500-ml conical flask; 170 ml of water were added, and the contents of the flask were boiled gently, with occasional swirling, until the sample had dissolved. The addition of ammonia, recommended by previous workers,[1,2] was found to be unnecessary. After the solution had been cooled to about 50° C, 50 ml of concentrated hydrochloric acid, 1 ml of potassium metabisulphite solution and 1 ml of potassium iodide solution were added, and the whole was set aside for 15 minutes. The solution was transferred to a 500-ml separating funnel, saturated with chloroform, extracted twice with a solution of diethylammonium diethyldithiocarbamate in chloroform and finally shaken with chloroform. The combined organic layers were scrubbed with a solution of oxalic and hydrochloric acids in a 100-ml separating funnel, transferred to a 100-ml conical flask and oxidised with nitric and perchloric acids. Evaporation with hydrochloric acid removed any last traces of germanium, and finally the volatile acids were removed by heating the solution until fumes of sulphuric acid were evolved.

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The probable sources of error in the separation described above include loss of arsenic on dissolution of the sample, incomplete extraction of the arsenic, loss of arsenic on scrubbing the chloroform extract, loss of arsenic during the final stages of fuming and introduction of arsenic from the reagents, especially the oxalic and hydrochloric acids.

To study the efficiency of the separation, a series of experiments was carried out on a radioisotope of arsenic. The initial activity of the isotope was such that 0·25 μg of arsenic-76 in 10 ml of solution gave about 35,000 counts per minute. Measurements were made with a M6H liquid counter (20th Century Electronics Ltd.) and a Panax Ratemeter type 5054. To determine the activity of a solution, its volume was measured, and 10 ml were transferred by means of a measuring cylinder to the liquid counter; 2 minutes were allowed for the ratemeter to stabilise, and nine readings were taken at 15-second intervals. The product of the volume of the solution and the average count rate (corrected for lost counts) was taken as a measure of the arsenic in the solution. No correction was applied for variations in density or composition of the solutions being measured.

The initial experiments, performed by adding quinquivalent arsenic-76 as sodium arsenate, before dissolution of the germanium dioxide in oxalic acid, confirmed that no loss of arsenic occurred when the germanium dioxide was dissolved and that about 99 per cent. of the tervalent arsenic was extracted into the chloroform. Quinquivalent arsenic was not extracted, and reduction to the tervalent state by sulphite and iodide did not take place in the absence of hydrochloric acid under the conditions described above. When the chloroform extract was scrubbed with hydrochloric and oxalic acids to reduce its germanium content, about 12 per cent. of the arsenic returned to the aqueous phase. If hydrochloric acid was omitted from the scrubbing solution the loss of arsenic was reduced to 3 per cent. This was considered satisfactory.

DETERMINATION OF ARSENIC—

Of the methods available for determining submicrogram amounts of arsenic, that described by Liederman, Bowen and Milner[7] appeared the best for further study. In this method the arsenic in dilute sulphuric acid is reduced to the tervalent state with stannous chloride and potassium iodide; 5 g of zinc are added, and the evolved hydrogen, containing all of the arsenic as arsine, is bubbled through a solution of silver diethyldithiocarbamate in pyridine. After hydrogen has been bubbled through the solution for 45 minutes, the optical density is measured at 540 mμ, and the red colour of the complex formed is proportional to the arsenic content of the solution in the range 0 to 5 μg of arsenic.[6] Antimony, which accompanies arsenic through the whole procedure, forms a colour with an absorption peak at 510 mμ. The optical density of antimony at 540 mμ is 8 per cent. of that of arsenic under these conditions, but the traces of antimony present in the sample of germanium dioxide are too small to contribute measurably to the final colour.

The apparatus used for the evolution and absorption of arsine is described by Crawford, Palmer and Wood.[8] It consists of a 50-ml conical flask with a B19 neck, an inverted U connecting tube with a B19 cone at one end and a B7 cone at the other, a narrow gas-delivery tube, with a B7 socket at one end, and a glass helix about 3 inches long fitting outside the delivery tube and inside a flat-bottomed glass cylinder. Before use, the bulb below the B7 socket of the bubbler tube is plugged with cotton-wool impregnated with lead acetate. By pipette, 3 ml of silver diethyldithiocarbamate solution in pyridine are put into the absorber cylinder, the gas-delivery tube and the helix are inserted, and the apparatus is assembled. When the apparatus was new no lubricant was used on the ground-glass surfaces; after several months it was necessary to moisten the joints with a small amount of glycerol.

INTERFERENCE BY GERMANIUM—

There is no reference in the literature to the effect of germanium on the determination of arsenic. If any germanium is present in the evolution solution some will be evolved as germane, which forms an unstable red complex with the silver compound. The position of the optical-density peak around 490 mμ changes slowly, and the colour intensity is reduced by 90 per cent. in about 5 hours. Germanium also interferes by preventing complete evolution of arsenic as arsine. This inhibition is not reproducible, and the addition of oxalic acid to the evolution solution (to complex the germanium) does not completely suppress the interference.[1] It is therefore essential to reduce the germanium content to the submicrogram level before the evolution of arsenic is carried out. If significant amounts of germanium are present in the solution, the optical density of the pyridine solution at 490 mμ will be greater than that at 540 mμ; should this be so, the determination must be repeated.

PURIFICATION OF OXALIC ACID—

Payne[1] recommended one re-crystallisation from hydrochloric acid and then two from water to purify the oxalic acid. This was tried, but it was found that traces of hydrochloric acid in the oxalic acid gave rise to loss of arsenic during dissolution of the sample. Radioactive arsenic-76 was used to compare this mode of purification with triple re-crystallisation from water. Measurement of the activity of the mother liquor after each re-crystallisation step, whether from acid or water, showed that about 85 per cent. of the arsenic present was removed with each operation, so re-crystallisation from water was adopted as the method of purification.

EVOLUTION CONDITIONS—

The largest source of arsenic from the reagents was the zinc metal used in the evolution step. It was observed that the arsenic blank values rose with an increase in the amount of zinc dissolved, and this in its turn increased with the time allowed for evolution. An experiment was carried out to determine the time after which an acceptable proportion of the arsenic had been evolved as arsine and absorbed in the pyridine solution. Optical-density measurements were made at 15-minute intervals on absorption solutions from standards containing 0·5 and 2·5 μg of arsenic. For the former, about 98 per cent. of the arsenic was evolved within 30 minutes. The latter sample took 45 minutes for 98 per cent. to be evolved, though 95 per cent. was liberated after half an hour. As, in the proposed determination, less than 0·5 μg of arsenic is to be evolved, the evolution time was fixed at 30 minutes. The weight of zinc used for the evolution was reduced to 2 g with no change in the optical density produced by 0·5 μg of arsenic. This had the dual effect of diminishing the blank value and conserving the selected batch of low-arsenic zinc.

METHOD

APPARATUS—

As well as normal laboratory glassware, three sets of apparatus for the evolution and absorption of arsine are required. The apparatus is described by Crawford, Palmer and Wood.[8]

The glassware must be cleaned with chromic acid cleaning solution. Ensure that the taps and stoppers of the separating funnels fit well and that the stems and taps are dry. Dry them with acetone and a current of air if necessary. Reserve the flasks and funnels for determinations of trace amounts of arsenic.

Exelo piston pipettes can conveniently be used to transfer the organic solutions.

REAGENTS—

All reagents must be of recognised analytical grade unless otherwise stated.

Water once distilled from a Pyrex-glass still is satisfactory.

Standard arsenic solution—Dissolve 0·132 g of arsenic trioxide in 2 ml of N sodium hydroxide in a covered beaker. Add 10 ml of water and 2 ml of 100-volume hydrogen peroxide, and set aside until effervescence ceases. Dilute the solution to 1000 ml. From this stock solution (0·100 mg arsenic per ml), prepare daily a fresh working solution containing 0·25 μg of arsenic per ml.

Oxalic acid—Batches having an extremely low arsenic content have been obtained. If the arsenic level exceeds 0·0025 p.p.m. (0·05 μg of arsenic in 20 g) re-crystallise twice or three times from water.

Hydrochloric acid—Examine all available samples for arsenic content, and reserve the best batch for this determination.

Potassium iodide solution, 10 per cent. w/v, aqueous—Discard after 2 weeks.

Potassium metabisulphite solution, 5 per cent. w/v, aqueous—Discard after 2 weeks.

Diethylammonium diethyldithiocarbamate solution, 0·25 per cent. w/v in chloroform.

Oxalic acid solution, 5 per cent. w/v, aqueous.

Nitric - perchloric acid (3 + 1) *v/v*—Mix 3 volumes of concentrated nitric acid with one volume of 72 per cent. perchloric acid.

Diluted hydrochloric acid—Dilute the concentrated acid with an equal volume of water, and add 1 per cent. v/v of concentrated nitric acid.

Mixed acid—Add 2 volumes of concentrated sulphuric acid (low in arsenic) and 1 volume of 72 per cent. perchloric acid to 1 volume of concentrated nitric acid.

Stannous chloride solution, 10 per cent. w/v—Dissolve stannous chloride, $SnCl_2.2H_2O$, in diluted hydrochloric acid (1 + 2). Discard after 2 weeks.

Silver diethyldithiocarbamate[7]—This can be purchased from the Fisher Scientific Co., or prepared as described below. Prepare two solutions, one containing 1·7 g of silver nitrate in 100 ml of water and the other containing 2·3 g of sodium diethyldithiocarbamate in 100 ml of water. Cool to below 20° C, and mix slowly, with stirring. Filter off the lemon-yellow product, and wash thoroughly with distilled water. Dry in a vacuum desiccator below 30° C. The dry salt is stable for at least 6 months.

Silver diethyldithiocarbamate solution, 0·5 per cent. w/v in pyridine.

Zinc—Select a batch of granulated zinc with an arsenic content below 0·05 p.p.m.

Cotton-wool—Impregnated with lead acetate (obtained from British Drug Houses Ltd.)

PROCEDURE—

Carry out one determination of arsenic in the reagents with each sample or group of samples.

Heat 5 g of germanium dioxide, 20 g of oxalic acid and 150 ml of water in a 500-ml conical flask, with occasional swirling, until the solution is clear. Set aside to cool, add 30 ml of concentrated hydrochloric acid, and adjust the temperature to between 60° and 65° C. Add 2 ml of potassium iodide solution and 1 ml of potassium metabisulphite solution to the contents of the flask, and set aside for 15 minutes to permit the reduction of quinquivalent arsenic to the tervalent state.

Transfer the contents of the flask to a 500-ml separating funnel containing 5 ml of diethylammonium diethyldithiocarbamate solution and 5 ml of chloroform. Swirl the funnel carefully, and loosen the stopper (not the tap) to release the pressure; repeat this operation until the whole of the funnel is at the same temperature, and then shake the funnel vigorously for 40 seconds. Allow the layers to separate, and then run the lower layer slowly into a 100-ml separating funnel, great care being taken that no aqueous phase enters the tap. Add 5 ml of carbamate solution to the contents of the 500-ml separating funnel, and repeat the pressure releasing operation, the extraction and separation. Finally, shake the germanium oxalate solution with 5 ml of chloroform, separate, and combine with the other extracts.

Shake the combined chloroform phases with 10 ml of 5 per cent. oxalic acid solution for 40 seconds to remove any entrained germanium. Allow the layers to separate completely, and run the lower layer into a 100-ml conical flask. Add 2 ml of nitric - perchloric acid to the contents of the conical flask, evaporate the chloroform carefully, and then heat the flask until fumes of perchloric acid appear. Set aside to cool, add 2 ml of diluted hydrochloric acid, and repeat the evaporation to fumes. This treatment removes the last traces of germanium. Finally, add 4 ml of mixed acid to the contents of the conical flask, and heat to fumes of sulphuric acid. During the heating with mixed acid the nitric acid is evaporated first; fumes of perchloric acid are evolved and the solution turns yellow, and after the perchloric acid has been removed the solution is almost colourless. Continue the heating until sulphuric acid is condensing 1 cm above the bottom of the flask. Remove the flask, and set it aside to cool to room temperature; add 2 ml of diluted sulphuric acid (1 + 1) and 12 ml of water, and transfer the solution to the 50-ml conical flask of the arsine-evolution apparatus. Add 1 ml of potassium iodide and 1 ml of stannous chloride solution to the contents of this flask, and set it aside for 15 minutes. Fit the bubbler tube with lead acetate impregnated cotton-wool, and with the aid of a piston pipette transfer 3·0 ml of silver diethyldithiocarbamate solution to the bubbler. After 15 minutes add between 2 and 2·3 g of zinc to the contents of the conical flask, and connect the flask to the apparatus. Allow hydrogen to be evolved for 30 minutes, disconnect the apparatus, and transfer the pyridine solution to a 1-cm cell. Measure the optical density spectrophotometrically against the pure silver diethyldithiocarbamate solution at 540 mμ with a slit width of 0·1 mm. Subtract the optical density produced by the reagents alone from the figure so obtained, and determine the arsenic content of the sample by comparing it with a standard solution taken through the same procedure. Prepare the standard solution by adding 0·5 μg of quinquivalent arsenic, as sodium arsenate, to 5 g of germanium dioxide, and calculate the optical density due to the recovered arsenic. If the standard arsenic is in a hydrochloric acid solution, the acid must be added after the dissolution of sample in oxalic acid, or loss of arsenic will result.

NOTES—

1. Obtain the cell blank value by measuring each cell in turn against the reference cell with silver diethyldithiocarbamate solution in each. Subtract the cell blank value from the measured optical density of the sample. Empty the cells with a piston pipette. If the faces of the cells are wetted or touched repeat the cell blank measurement.

2. If the results are higher than expected and the presence of germanium in the evolution flask is suspected, measure the optical density of the coloured solution at 490 mμ. If this figure exceeds the optical density at 540 mμ, repeat the determination.

3. Depending on the thermal history of the sample, complete dissolution in oxalic acid may not be attained within 50 minutes. All of the samples examined so far have either dissolved completely within 30 minutes, or have had about 5 per cent. (estimated) remain undissolved after 50 minutes. The undissolved sample can be ignored in the determination by permitting it to settle during the 15-minute reduction period and decanting the supernatant solution into the 500-ml separating funnel.

Most of the remaining germanium dioxide collects at the liquid interface, and if the chloroform phase is run through the tap slowly (say over a period of 1 minute) no solid passes through the tap. It is not essential to plug the stem of the tap funnel with glass-wool.

4. During the 15-minute reduction period the temperature of the solution drops from about 60° to 45° C. If the solution cools below about 35° C, crystallisation of oxalic acid from the reagent blank solution will begin with consequent blocking of the stem above the tap of the funnel. For convenience, therefore, the extraction should be effected within 15 minutes of completion of reduction.

5. Some batches of silver diethyldithiocarbamate have been prepared that showed a variable reaction to small amounts of arsenic. Amounts up to 1 μg of arsenic give no reaction with these batches, although above this "threshold" the optical density per microgram of arsenic compares favourably with the good batches of reagent. The

TABLE I

RESULTS OF THE ANALYSIS OF 3 SAMPLES OF GERMANIUM DIOXIDE

		Optical density at 540 mμ of—				
			solution			
Sample No.	Solution	1-cm cell blank value	un-corrected	corrected	Optical density ratio of As in sample to As recovered from 0·5 μg	Arsenic content, p.p.m.
1	Reagent blank solution ..	−0·013	−0·009	0·004	0·0 to 0·042	<0·01
	5 g of germanium dioxide ..	−0·016	−0·013	0·003		
	5 g of germanium dioxide + 0·5 μg of arsenic ..	−0·015	0·030	0·045		
2	Reagent blank solution ..	0·005	0·018	0·013	0·007 to 0·037	0·02
	5 g of germanium dioxide ..	−0·007	0·013	0·020		
	5 g of germanium dioxide + 0·5 μg of arsenic ..	0·0	0·057	0·057		
3	Reagent blank solution ..	−0·001	0·013	0·014	0·011 to 0·039	0·03
	5 g of germanium dioxide ..	−0·011	0·014	0·025		
	5 g of germanium dioxide + 0·5 μg of arsenic ..	−0·003	0·061	0·064		

only variables in the preparation procedure that appeared to be significant were that there should be an excess of silver after the precipitation, and that the product should be dried thoroughly. Variations in the purity of the sodium diethyldithiocarbamate may also be important.

6. Arsine is slightly soluble in water. If the inside of the inverted U connecting tube has drops of water adhering to it (above the B19 cone) poor recoveries of arsenic frequently result. It has been found necessary to clean the connecting tube with cleaning solution, and to dry the tube so as to leave a hydrophilic surface. Drops of dilute acid spray are then able to drain back into the evolution flask.

A plug of cotton-wool impregnated with lead acetate is omitted from the B19 cone for the same reason, *i.e.*, spray from the hydrogen evolution wets the cotton-wool and often causes low results. The small plug in the B7 cone of the bubbler is sufficient to retain any traces of sulphide.

Three samples of germanium dioxide were analysed during one day; the method was exactly as described, except that 50 ml of concentrated hydrochloric acid were used in the reduction step before extraction. The results are shown in Table I.

Two further samples were analysed some months later exactly as described in the proposed procedure, *i.e.*, 30 ml of concentrated hydrochloric acid being used in the reduction step; the results are shown in Table II.

TABLE II

RESULTS OF THE ANALYSIS OF 2 SAMPLES OF GERMANIUM DIOXIDE

		Optical density at 540 mμ of—				
			solution			
Sample No.	Solution	1-cm cell blank value	un-corrected	corrected	Optical density ratio of As in sample to As recovered from 0·5 μg	Arsenic content, p.p.m.
4	Blank solution	−0·010	−0·003	0·007	0·009 to 0·051	0·02
	5 g of germanium dioxide..	−0·014	0·002	0·016		
	5 g of germanium dioxide + 0·5 μg of arsenic ..	−0·021	0·046	0·067		
5	Blank solution	0·006	0·009*	0·003*	<0·007 to 0·043	<0·015
	5 g of germanium dioxide..	0·012	0·022	0·010		
	5 g of germanium dioxide + 0·5 μg of arsenic ..	0·001	0·054	0·053		

* Represents that part of the total blank value contributed by the reagents added for oxidising the chloroform extracts and by the reagents added subsequently.

DISCUSSION OF THE METHOD

The sample dissolution and arsenic extraction described are extremely satisfactory. One possible improvement would be to find a method of reducing quinquivalent arsenic involving reagents that could be easily purified. However, the batches of reagents used in this work have contributed extremely little arsenic to the total blank value.

The spread in the values of the reagent blank solution (0·004 to 0·014 units) in samples 1 to 4 may be caused partly by non-reproducibility in the optical-density readings (about 0·002 units), but may also be caused by inhomogeneous distribution of arsenic in the zinc. There is also a considerable variation in the amount of granulated zinc left undissolved at the end of the 30-minute evolution period. If zinc chippings or borings of more uniform thickness were easily obtainable it would be possible to reduce the spread of the blank value. Strictly, an average reagent blank value (in this instance about 0·009) should be subtracted from the optical-density readings for the sample. The difference between the average reagent blank value and the extreme blank values is a measure of the error in the determination, which for this batch of zinc is about ±0·005 optical-density units, *i.e.*, ±0·05 μg of arsenic.

The agreement between the optical densities of the 0·5 μg of reserved arsenic (0·041, 0·037, 0·039, 0·051, 0·043) is satisfactory considering the grade of zinc used. Sample 4 inadvertently had a 40-minute evolution period, which may partly account for its high value.

It is difficult to pin-point the reasons for the wide variations in the cell blank values. The cells were cleaned with cleaning mixture before samples 1 and 4 were measured, and were never handled during measurements on samples 1 to 3 or 4 and 5. The changes in optical density of the cells during each of the 2 days were probably caused by the formation of salts of pyridine (*e.g.*, the hydrochloride) by sublimation on the outer faces of the cell. The magnitude of the cell blank values shows the need for the extra set of measurements before each group of sample optical-density measurements.

The variables that must be reconciled to obtain the conditions for the most accurate results from the evolution step are the arsenic content, the weight and the surface area of the zinc, the amount and concentration of the dilute sulphuric acid used, the evolution time and the percentage of arsenic converted to arsine, the volume of absorption solution and the optical path length. As granulated zinc was used, the differences in the thickness of the granules was considerable and difficult to take into consideration. The batch of zinc with the lowest arsenic content was selected from those available. As a result of the experiment described above the evolution time was fixed at 30 minutes.

Most of the published work suggests the use of 5 g of zinc; to diminish the blank value this amount was reduced first to 3 g and later to 2 g, with a corresponding lowering in the amount of sulphuric acid used. This reduction involves a decrease in the range over which Beer's law is obeyed. With 3 g of zinc the optical density per microgram of arsenic stayed constant up to 5 μg, but with 2 g of zinc the value dropped above 1 μg. The amount of sulphuric acid used is sufficient to react with all the zinc and provide a 50 per cent. excess; its dilution permits a satisfactory rate of hydrogen evolution. Three millilitres of pyridine solution used in the gas absorber are sufficient to cover the helix and so provide a long gas-to-liquid contact time. That the absorption reaction is rapid is shown by the frequent appearance of an intensely coloured red compound inside the inner bubbler tube where the glass had been wetted by the absorbent. This substance must be dissolved in the absorption solution before optical-density measurements are taken. The bubbler was designed for the absorption of arsine in iodine solution[8]; it is possible that a smaller volume of absorbent will suffice for silver diethyldithiocarbamate in pyridine. A cell of longer path length per unit volume would decrease the lower detectable limit of arsenic, although a good grade of zinc is necessary for any large improvement in sensitivity.

FURTHER APPLICATIONS—

To extend the method to the analysis of other materials the factors detailed below must be considered. Milligram amounts of copper, nickel and cobalt interfere with the evolution of arsine,[11] and traces of germanium and antimony are reduced to germane and stibine, which form coloured complexes with silver diethyldithiocarbamate. Arsenic, together with antimony and tin, can be separated from milligram amounts of the elements mentioned above, and many more, by Wyatt's procedure.[10] In this procedure an oxidised solution containing quinquivalent arsenic and antimony and quadrivalent tin is extracted with diethylammonium diethyldithiocarbamate; the extracted solution, adjusted to 2 N in hydrochloric acid, is reduced with potassium iodide and potassium metabisulphite, and is re-extracted. Traces of iron may accompany the arsenic, antimony and tin, but these will be insufficient to interfere with the evolution of arsine.

Antimony thus remains the only interfering element. Work in this laboratory has shown that the separation of antimony from arsenic can be effected by reduction of the antimony with hydrazine sulphate and heating to fumes of sulphuric acid, and then extraction of the antimony with cupferron.

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The Detection and Determination of Glycerol in Tobacco

The Use of Paper and Cellulose-column Chromatography for determining Glycerol in the Presence of Sugars

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A simple method of extraction and a sensitive paper-chromatographic procedure for the identification of glycerol, and of propylene glycol, which occasionally accompanies it in tobacco samples, are described.

For quantitative determination of glycerol, a method has been developed in which the glycerol is effectively separated from sugars on a column of cellulose; a layer of activated charcoal at the top of the column removes other tobacco materials, which would interfere with the subsequent determination of the glycerol by oxidation with periodate.

CUSTOMS Regulations in this country prohibit the sale of cigarettes containing added glycerol, although its addition is permitted in some other countries.

The method for determining glycerol in tobacco proposed by Chapman[1] some years ago has been considered to be the only method capable of providing accurate results, but it has the disadvantage of being extremely time-consuming and inconvenient for routine control purposes; the method outlined in the "Tobacco" monograph of Thorpe's Chemical Dictionary[2] has been in use for some years in the Laboratory of the Government Chemist. In this procedure, which is known to yield incomplete recovery of the glycerol present, the glycerol is separated by passing steam into the tobacco heated to 130° C under low pressure.

The qualitative paper-chromatographic procedure described in this paper serves as a preliminary sorting test; when the presence of added glycerol is indicated, the amount present can be determined quantitatively by the column procedure. When propylene glycol is present, it emerges from the column in the same eluate as the glycerol, and further investigatory work is continuing in order to provide an extension to the method so that both glycerol and propylene glycol can be determined accurately in tobacco.

QUALITATIVE PAPER-CHROMATOGRAPHIC TEST FOR GLYCEROL IN TOBACCO—

As a running solution for use in the paper chromatography of glycols and other polyhydric alcohols, solutions containing n-butanol have been recommended, *e.g.*,[3,4,5]; we prefer water-saturated n-butanol for separations by both ascending- and descending-solvent chromatography. Ammoniacal silver nitrate is used for developing the spots, the most satisfactory definition being obtained when the papers are impregnated with silver nitrate before the chromatograms are run and exposed to an atmosphere of ammonia immediately on removal from the tank.

Simple extraction of tobacco with water, and then, possibly, with solvent, is attractive as a means of obtaining a suitably concentrated solution for spotting on a chromatographic paper; however, when such a procedure is employed, interference from other materials extracted from some types of tobacco masks appreciable amounts of glycerol, as shown in Fig. 1 (No. 1). By mixing the tobacco with carbon during the extraction, some of the interfering materials were removed and a clearer solution was obtained for overspotting, but the interference from salts was not effectively removed (see Fig. 1, No. 2). By mixing the tobacco with diethylaminoethylcellulose powder the glycerol spots were satisfactorily isolated; this treatment was effective even when only traces of glycerol were present (see Fig. 1, Nos. 3 and 6). Acetone is the most convenient solvent for use in this procedure.

As a final washing solution after development of the chromatogram, it is considered that the use of 10 per cent. v/v ammonium hydroxide in the proportion recommended yields slightly more distinct spots than does sodium thiosulphate solution.

Fig. 1 shows the spots from a standard solution (No. 5) and also from a tobacco containing 1 per cent. of added glycerol (No. 4).

PAPER-CHROMATOGRAPHIC METHOD FOR DETECTING GLYCEROL AND PROPYLENE GLYCOL IN TOBACCO

REAGENTS—

Charcoal powder—For decolourising purposes, activated, washed with acid (obtainable from the British Drug Houses Ltd.).

Diethylaminoethylcellulose powder—Whatman D.E.50.

Dilute standard solution of glycerol and propylene glycol—0·1 per cent. w/v glycerol and 0·2 per cent. w/v propylene glycol in acetone containing approximately 25 per cent. of water. This solution should be freshly prepared, and is conveniently obtained from a stronger aqueous stock solution of glycerol and propylene glycol diluted with acetone.

Silver nitrate solution in acetone—Dissolve 1 g of silver nitrate in 6 ml of water, and dilute to 50 ml with acetone. The solution must be freshly prepared immediately before use.

Ammonium hydroxide solution—A 10 per cent. v/v solution of ammonium hydroxide, sp.gr. 0·880, in water.

PROCEDURE—

Add 3 ml of distilled water to 1 g of the tobacco contained in a small beaker, and stir for 5 minutes with a pointed glass rod; use a pressing action to ensure that the water penetrates into the tobacco. Add 10 ml of acetone, and mix thoroughly. Add 1·5 g of the activated charcoal and 1·5 g of diethylaminoethylcellulose powder, stir well for about 1 minute to ensure homogeneity, and then with a flat spatula press out a small amount of solution, approximately 3 ml, into a small specimen tube. Insert the stopper in the tube, and set aside for a short time until some clear supernatant liquid separates; meanwhile, prepare the chromatography paper.

Overspot the extract from the tobacco five times on to Whatman No. 1 chromatography paper, and allow a short time for drying (at room temperature) after each spot has been applied. Each spot should contain approximately 5 μl of solution, and the outside spots should be at least 1½ inches from the edges of the paper.

A spot of dilute standard solution of glycerol and propylene glycol should be included every time a paper is prepared.

When the spots have dried, impregnate the paper with silver nitrate by rapidly dipping it in silver nitrate solution in acetone. Allow the acetone to evaporate at room temperature in subdued light, and run the chromatogram, with ascending or descending solvent according to preference, in a tank protected from daylight, overnight in water-saturated n-butanol. Satisfactory separations can also be obtained with a short 3 to 4 hour descending-solvent run. (See Note 2.)

After the chromatogram has been run, rapidly transfer the wet paper to a tank containing ammonia. Close the tank, keep the paper in it for 3 minutes, and then dry the paper in still air at room temperature, preferably in subdued light, for not more than 30 minutes; reverse its position after 10 to 15 minutes. Then heat the paper in an oven at 95° to 100° C for approximately 15 minutes, and wash with 200 ml of 10 per cent. ammonium hydroxide and then under running tap-water for 5 minutes.

A spot in the glycerol position from tobacco containing no added glycerol will be visible on the chromatograms. Calculated from the concentration of the tobacco extract, *i.e.*, 1 g of tobacco in 13 ml of solution overspotted 5 times, the standard comparison spot of 0·1 per cent. glycerol spotted once corresponds to the maximum "natural" glycerol spot likely to be encountered. Any spot from the tobacco of less intensity than the standard should be ignored; a heavier spot is indicative of added glycerol, and quantitative column analysis should be carried out.

There should be no spot from tobacco in the propylene glycol position.

NOTES—

1. Glycols slowly evaporate from the paper in draughts and at elevated temperatures; the papers must therefore be dried at all stages at room temperature, and draughts in fume cupboards must be avoided.
2. The water saturated n-butanol running solution slowly becomes contaminated with silver. It should be renewed and the container cleaned out when it has been used for approximately four runs.

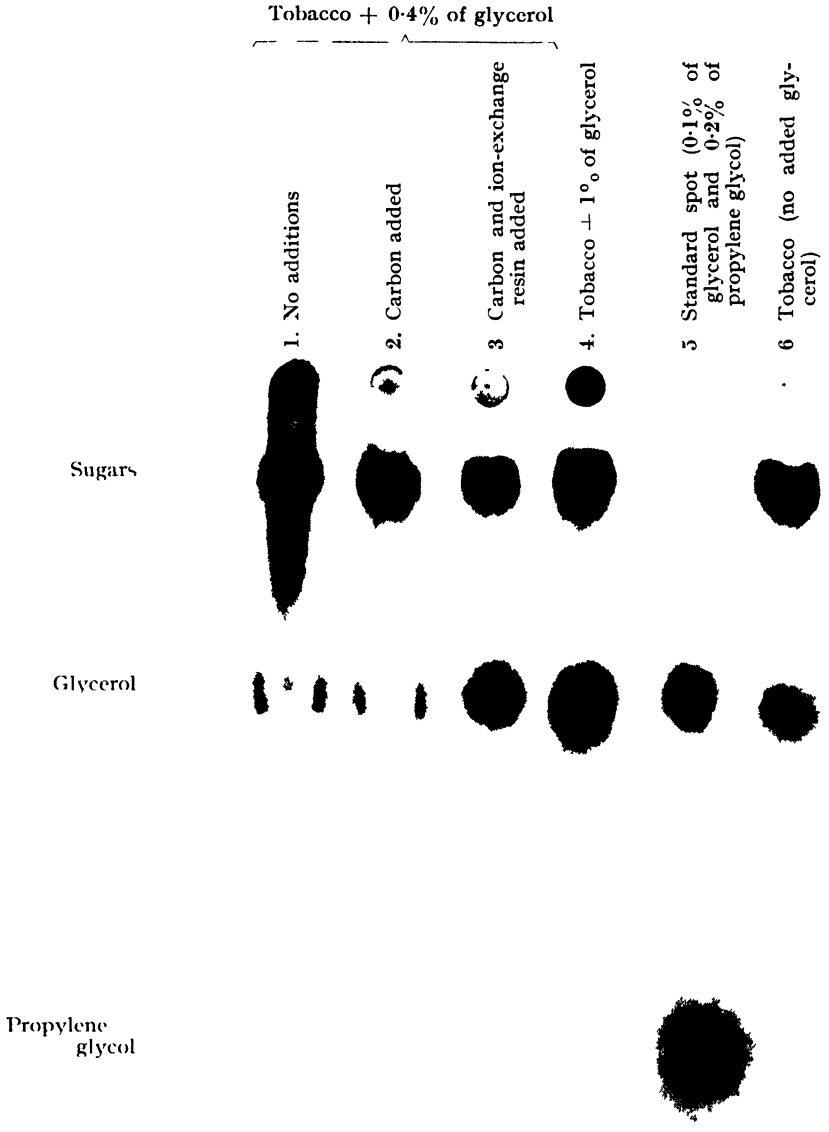

Fig 1. Chromatograms of tobacco and standards on Whatman No 1 paper, run in the machine direction, with ascending solvent for 17 hours at approximately 18° C

QUANTITATIVE DETERMINATION OF GLYCEROL BY CELLULOSE-COLUMN CHROMATOGRAPHY

Sporek and Williams[6] have described the determination of glycerol in its mixtures with sugars and the constituents of molasses, with alumina as adsorbent and acetone containing 5 per cent. of water and 0·05 per cent. of acetic acid as solvent. Sodium sulphite and sodium acetate are added to the sample solution to assist in the retention of sugars by the adsorbent. The solvent is later removed by evaporation from the eluate, and the glycerol is determined by oxidation with sodium metaperiodate and titration of the formic acid produced, thereby avoiding interference from other glycols present.

This method is most successful when the glycerol is present in materials less complex than tobacco. With tobacco we found that so much interfering material came through the column, even when it was modified by the addition of carbon, that the method was not considered suitable for our purpose. Moreover, traces of acetone are oxidised by periodate, and evaporation to ensure complete removal of acetone results in loss of propylene glycol.

Neish[7] separated glycerol from other materials present in fermentation solutions by elution with a mixture of benzene and n-butanol from a column of specially prepared Celite. The solvent was removed, and the formaldehyde formed from glycerol by oxidation with periodate was determined colorimetrically.

Our problem was somewhat different. We required a rapid accurate method for separating glycerol from appreciable amounts of sugars and from other tobacco materials, and a solvent for isolating glycerol that would allow oxidation with periodate to be carried out without the need for removing the solvent by evaporation. The special difficulties inherent in tobacco analysis were solved by using a two-layer column of cellulose powder and activated carbon. The preliminary extraction of glycerol was effected, without heating or evaporation, by stirring the sample first with a small volume of cold water and then with the solvent mixture. From experiments carried out with several solvents, we concluded that the most satisfactory and least obnoxious for our purpose was a mixture of two volumes of diethyl ether with one volume of ethanol.

In early experiments, excessive disturbance of the column was observed during elution. This disturbance was reduced as the width of the column was increased, but too wide a column gave insufficient separation of glycerol from sugars; the width recommended is the optimum for satisfactory separation. Occasionally, a column has shown some tendency to leave the sides of the tube, but no interference with the chromatographic separation was observed. The 5 g of carbon recommended is the amount we have found the most satisfactory for removing significant amounts of interfering materials from 1 g of tobacco and allowing solvent to run through the column at a suitable rate without the application of pressure or suction. The presence of the layer of carbon at the top also assists in maintaining the stability of the column.

Several variations of the method for determining glycerol by oxidation with periodate have been published since the oxidising action of periodates was first described by Malaprade.[8] Hartman's study of the procedure[9] contains a brief description of much of this work, and the accurate determination of glycerol by titration of the formic acid produced on oxidation with sodium metaperiodate is described in considerable detail by Erskine *et al.*[10] For our purpose we found that the simple procedure described below, based on total reduction of periodate, gives accurate results; removal of the solvent before oxidation is unnecessary provided sufficient water is added to the reaction mixture, and the addition of further water before the titration ensures that no preferential solution of iodine in the ether takes place. During the experimental work, we obtained duplicate titres agreeing to within 0·02 ml of 0·05 N sodium thiosulphate, although it is considered that titrating to the nearest 0·05 ml is sufficiently accurate for routine determinations.

SPECIAL APPARATUS—

Glass tubes for chromatography, 50 cm long and 2·5 cm internal diameter, widening out to approximately 4 cm diameter for the top 5 cm of length. A short length of 7-mm glass tubing is fused on to the bottom.

REAGENTS—

Ashless cellulose powder—Whatman standard grade.

Charcoal powder—For decolourising purposes, activated, washed with acid (obtainable from the British Drug Houses Ltd.).

Solvent mixture—Two parts by volume of diethyl ether mixed with 1 part by volume of 95 per cent. ethanol.

Potassium periodate solution—Dissolve 1·15 g of analytical-reagent grade potassium periodate in about 600 ml of distilled water containing 50 ml of 0·1 N sulphuric acid, warming if necessary, and dilute to 1 litre with distilled water.

Sodium thiosulphate, approximately 0·05 N.

Potassium iodide solution, approximately 10 *per cent. w/v., aqueous.*

Sulphuric acid, approximately 6 N.

Starch solution, approximately 1 *per cent., aqueous.*

PROCEDURE FOR SEPARATING THE GLYCEROL—

By pipette add 3 ml of distilled water to 1 g of the tobacco contained in a small beaker, and stir for 5 minutes with a small pointed glass rod; use a pressing action to ensure that the water penetrates into the tobacco. Add 10 ml of the solvent mixture, stir well, and set aside for about 20 minutes while preparing the column.

Place a small plug of absorbent cotton-wool inside the column, press well down, and moisten it with a little of the solvent mixture. Stir 30 g of the cellulose powder in a beaker with 200 ml of the solvent, and pour the mixture into the column as completely as possible in one operation. Any powder remaining in the beaker should be mixed with a small amount of solvent and added as rapidly as possible to the column before it has settled. When a layer of about 1 inch of solvent remains above the cellulose on the column, stir a mixture of 5 g of activated charcoal and 5 g of cellulose with 100 ml of solvent, and pour it into the column.

Allow the column to settle again, and in the meantime add 3·5 g of cellulose powder to the tobacco mixture in the beaker, and mix well. Occasionally, when dealing with fine cut tobacco, it may be necessary to add a little more cellulose in order to absorb all surplus liquid in the beaker. When a layer of about 2 inches of solvent mixture remains on the column, stopper the outlet with a short length of silicone rubber tubing closed by a short glass rod. Transfer the tobacco mixture quantitatively to the column, keeping sufficient solvent above the solid in the column to receive it, then unstopper the outlet, and place a 100-ml graduated flask underneath to receive the eluate. Allow the column to run until the head of liquid is reduced to about 2 mm above the tobacco, rinse the beaker with approximately 5 ml of the solvent, and transfer to the column; allow to drain down to a 2-mm head again, and repeat this procedure a further three times to ensure that all the material has entered the column. Reject the first 100 ml of eluate, and collect the glycerol into a further 500 ml of eluate in a graduated flask. At this stage it is permissible to keep a good "head" of solvent on the column, so that the flow is somewhat more rapid, and the 500 ml of eluate is collected in about 2 to 2½ hours.

PROCEDURE FOR OXIDISING THE GLYCEROL WITH PERIODATE—

By pipette transfer 50 ml of the eluate to a 500-ml conical flask having a ground-glass socket, add 100 ml of distilled water and then 25 ml of the potassium periodate solution from a pipette, mix, close the flask with a glass stopper, and set aside at room temperature in the dark for 40 minutes. Add 200 ml of distilled water, 10 ml of 20 per cent. potassium iodide solution and then 10 ml of 6 N sulphuric acid, mix, and titrate immediately with 0·05 N sodium thiosulphate from a 25-ml burette. Titrate as rapidly as possible, mixing well after each 1-ml addition of sodium thiosulphate solution. Add 1 ml of freshly prepared starch solution when about 1 ml from the end-point, stopper the flask, shake thoroughly, and complete the titration with frequent shaking until one drop discharges the colour. Carry out a blank titration at the same time on 50 ml of the solvent mixture. Duplicate titrations should agree to within 0·05 ml. If the titration difference between blank and sample exceeds 4·0 ml of 0·05 N sodium thiosulphate under the conditions recommended, the oxidation should be repeated on a smaller portion of the eluate.

1 ml of N sodium thiosulphate ≡ 0·023 g of glycerol.

Added glycerol = total glycerol (per cent.) — x (see Table II)

where x is the blank value determined on unadulterated tobacco of similar origin.

RECOVERY OF GLYCEROL FROM THE COLUMN

The volume of eluate collected adequately allows for the satisfactory recovery of glycerol unaccompanied by sugars over a temperature range of 15° to 30° C, *e.g.*, in the experiment illustrated by the histogram (Fig. 2), a mixture of 50 mg of glycerol with 100 mg each of sucrose, dextrose and laevulose was applied to the column, and the eluate was collected in fractions that were separately analysed for glycerol.

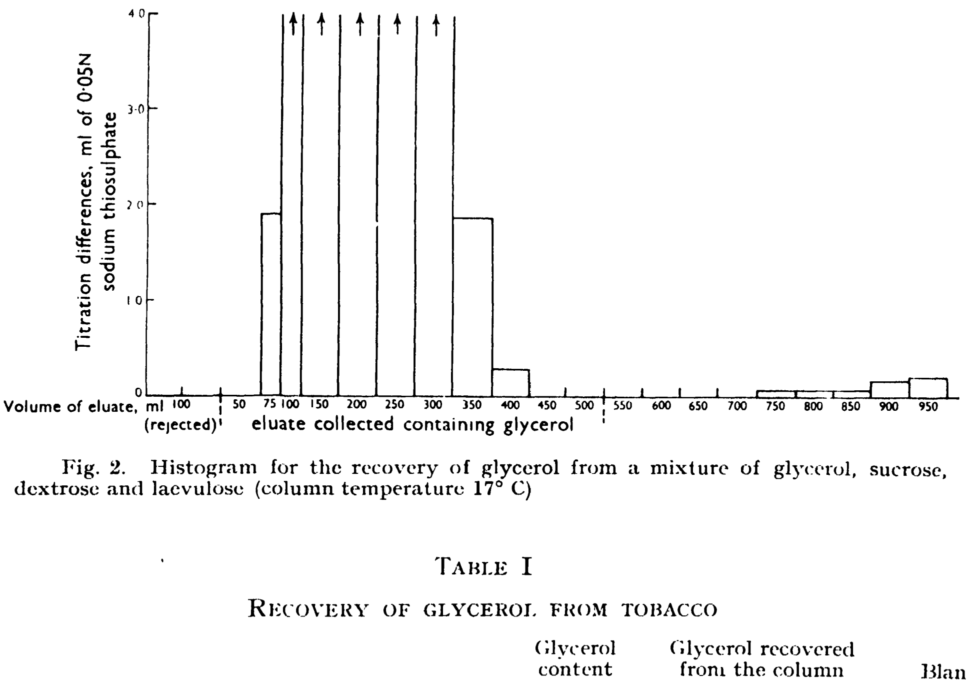

Fig. 2. Histogram for the recovery of glycerol from a mixture of glycerol, sucrose, dextrose and laevulose (column temperature 17° C)

TABLE I

RECOVERY OF GLYCEROL FROM TOBACCO

Experiment No.	Glycerol added	Glycerol content of the solution, %	Glycerol recovered from the column		Blank test on tobacco, %
			Cellulose, %	Tobacco, %	
1	1 ml of glycerol solution mixed with cellulose powder	3·0	3·0	- -	—
2	1 ml of glycerol solution + 100 mg each of dextrose and laevulose mixed with cellulose powder ..	1·9	1·9	—	- -
3	1 ml of glycerol solution + 200 mg of sucrose mixed with cellulose powder	3·9	3·9	—	—
4	1 ml of glycerol solution mixed with 1 g of tobacco	1·9	—	1·9	0·1
5		2·0	—	2·1	0·1
6		2·9	—	2·9	0·1
7		3·0	—	3·0	0·1
8		3·0	—	3·0	0·1
9		3·9	—	4·0	0·2
10		4·9	—	5·0	0·2
11	1 ml of glycerol solution dried into 1 g of tobacco	1·9	—	2·0	0·1
12		2·1	—	2·1	0·1
13	Blank solvent elution of column ..	—	No glycerol recovered		—

The results of various recovery experiments, including a blank experiment on the column, are shown in Table I. In each experiment glycerol was added to the sample as an accurately measured volume of an aqueous solution; at the same time an equal volume of the glycerol solution was diluted to 500 ml with solvent mixture, and the glycerol content of this control solution was determined by oxidation with periodate alongside the column eluate. Experiments 11 and 12 show the recovery of glycerol added to tobacco under conditions more severe then a manufacturer might be expected to employ: a measured volume of glycerol solution was mixed with 1 g of tobacco and heated in an oven at approximately 100° C for 1½ hours; the glycerol was then eluted from the column and determined alongside a control solution as in the other recovery experiments. In the recovery experiments from tobacco a determination of glycerol was carried out on the tobacco used without any added glycerol (see column 5).

Glycerol content of tobacco

The first samples of imported cigarettes containing glycerol examined by this method gave results unexpectedly higher than those obtained by the vacuum steam-distillation procedure. In one experiment a recovery of over three times as much was obtained, and it was considered advisable to establish that no appreciable interference might be encountered when dealing with a tobacco from a foreign source. Samples of imported unmanufactured tobacco leaf and cigarettes containing no added glycerol originating from several different sources were examined, and the results (see Table II) range from 0·4 per cent. of apparent glycerol down to a negligible amount, but are mostly about 0·2 per cent.

Comparative figures obtained by the qualitative paper-chromatographic procedure, in which the density of spots appearing in the glycerol position were compared with glycerol standards, are shown in column 4 of Table II. A typical spot, equivalent to 0·2 per cent. of glycerol, is shown in Fig. 1 (No. 6).

Table II

Results by column and paper chromatography for tobacco containing no added glycerol

Sample No.	Country of origin	Apparent glycerol content by— column procedure, %	Apparent glycerol content by— paper procedure, % (approx.)
Unmanufactured tobacco—			
1	U.S.A.	0·2	<0·1
2		0·2	<0·1
3		0·2	<0·1
4		0·2	0·1
5		0·2	0·1
6		0·3	0·1
7		0·2	0·1
8		0·2	0·1
9	India	0·3	0·1
10		0·2	0·1
11		0·2	0·1
12		0·3	0·1
13		0·2	0·1
14		0·2	0·1
15		0·2	0·1
16		0·2	<0·1
17	Rhodesia	0·2	<0·1
18		0·2	0·1
19		0·1	0·1
20		0·2	0·1
21		0·2	0·1
22		0·2	<0·1
23		0·1	<0·1
24		0·1	<0·1

Sample No.	Country of manufacture	Apparent glycerol content by— column procedure, %	Apparent glycerol content by— paper procedure, % (approx.)
Cigarettes—			
1	U.S.A.	0·2	0·1
2		0·2	0·1
3	India	0·2	<0·1
4	United Kingdom	0·1	0·1
5		0·2	0·1
6		0·1	0·1
7		0·2	<0·1
8		0·1	<0·1
9	France	<0·1	<0·1
10	Cuba	0·1	<0·1
11		0·1	<0·1
12	Italy	0·2	0·1
13	Turkey	0·4	
14	Russia	0·3	0·2
15	Greece	0·2	0·1
16	Egypt	0·4	0·2

It can be seen from Table II that there is usually a slight discrepancy between the results by the column and by the paper-chromatographic procedures, and, as diethylaminoethylcellulose has been successfully used for removing salts before paper chromatography, experiments were carried out in which various amounts of the ion-exchange resin were incorporated in the cellulose - carbon column. Some reduction of the figures shown (less than 0·1 per cent.) was obtained, but the inclusion of ion-exchange resin in the column is considered unnecessary, since the extremely small differences involved would not be detected within the limits of accuracy of titration considered to be the most suitable for routine work. Reductions of the same order were obtained when the initial aqueous extracting solution was rendered alkaline with sodium hydroxide.

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The Determination of Free Sulphur Dioxide in Soft Drinks by a Desorption and Trapping Method

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Experiments have shown that, under specified conditions, the desorption of free sulphur dioxide in soft drinks is much faster than the dissociation of combined sulphur dioxide. This principle has been used in developing a method for determining free sulphur dioxide as an alternative to methods involving direct titration of the sample with iodine. The proposed method does not suffer from drifting end-points or high blank values, which are often encountered in direct titration methods. Free sulphur dioxide is desorbed at room temperature and at pH 1 to 2 by a rapid stream of oxygen-free nitrogen. For 50 p.p.m. or more of free sulphur dioxide, it is trapped in in alkaline glycerol and the determination completed iodimetrically. Lower concentrations are trapped in sodium tetrachloromercurate reagent, and the determination is completed colorimetrically. With slight modification the method has been applied to samples having high and low concentrations of sulphur dioxide, samples having high and low contents of solids and to coloured and colourless samples. When the proposed method was applied to various soft drinks the results agreed satisfactorily with those obtained by a direct titration method.

THE classical method for determining free sulphur dioxide in fruit juices and soft drinks consists of direct titration of the sample with iodine, and then titration of a second portion with iodine in the presence of a carbonyl compound, the difference in titres being due to free sulphur dioxide. The chief disadvantages of this type of method are drifting end-points in one or both titrations, a relatively high second or blank titre compared with the titre difference (particularly if the ascorbic acid content of the sample is high) and difficulty in assessing the end-point with coloured samples. To overcome two of these disadvantages, Vas[1] showed that, at pH values of less than 2, the greater stability of glucose bisulphite leads to sharper end-points, and these can be further improved by an electrometric method described by Ingram,[2] which also permits determination in coloured samples.

Nevertheless, results from this type of method, which depends on the difference between the two iodine titrations, both of which are carried out in the presence of a large amount of reducing or potentially reducing organic material, are bound to depend to some extent on the personal judgment of the analyst. This could be avoided if it were possible to remove free sulphur dioxide from the sample in a similar way to the removal of total sulphur dioxide in the Monier-Williams method.[3]

Previous experience in desorbing dissolved oxygen from soft drinks had indicated that the desorption of sulphur dioxide from small volumes, though not as fast as that of oxygen, was still fairly rapid. Further, examination of the dissociation constants of glucose bisulphite showed that, at pH 1 to 2 and under conditions likely to be found in soft drinks, the extent of dissociation in 30 minutes would be extremely small; if this was also true for other sulphur dioxide complexes found in drinks, then it should be possible to complete the desorption of free sulphur dioxide before any effective dissociation of combined sulphur dioxide occurred.

EXPERIMENTAL

PRELIMINARY EXPERIMENTS—

The first experiment was carried out in a pear-shaped polarographic cell described by Lloyd and Parkinson,[4] which can be used to follow the rate of desorption of certain dissolved gases. It was found that approximately 40 p.p.m. of free sulphur dioxide could be desorbed at room temperature from a dextrose solution in acetate buffer, acidified to a pH value of 1 to 2, by a rapid stream of oxygen-free nitrogen at room temperature in about 5 minutes. Subsequent dissociation of combined sulphur dioxide was found to be negligible, and blank titres were extremely small.

These results were so promising, even when the near-ideal desorption conditions in the pear-shaped cell were taken into account, that an apparatus of the type shown in Fig. 1 was made. With this apparatus, a similar experiment to that in the pear-shaped polarographic cell was carried out, but the desorbed sulphur dioxide was trapped and determined. The choice of trapping reagent and subsequent method of determination was made at this stage. The use of neutral hydrogen peroxide in the traps and then titration with standard sodium hydroxide solution did not give sufficiently sharp end-points, even when screened methyl orange was used as indicator. By trapping in alkaline glycerol, however, the subsequent determination could satisfactorily be made by Aulenback and Balmat's[5] polarographic method or alternatively by acidification and iodimetry. A colorimetric method, in which were used the reagents described by Beetch and Oetzel,[6] was found to be particularly suitable for small amounts of trapped sulphur dioxide. In our experiments, to avoid the use of a comparatively expensive polarograph, the iodimetric method was adopted for high concentrations and the colorimetric method for low concentrations of free sulphur dioxide.

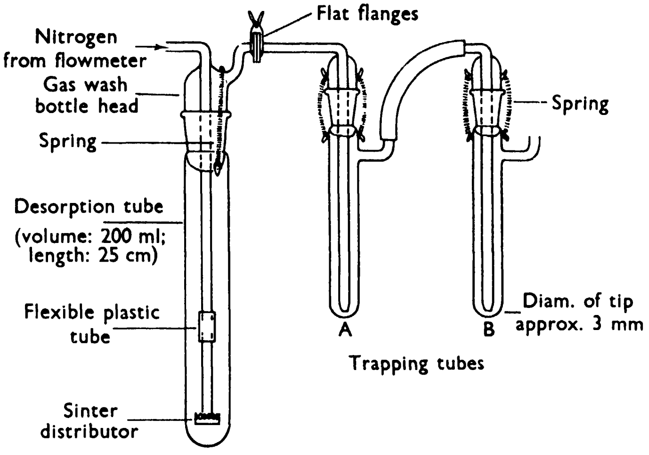

Fig. 1. Apparatus for determining free sulphur dioxide by a desorption and trapping method

Further preliminary experiments were carried out to study the application of the method to various sugar solutions and a sucrose - ascorbic acid solution, all of which had been sulphited and stored at pH 3 until equilibrium between free and combined sulphur dioxide was attained. Free sulphur dioxide was simultaneously determined by direct iodimetry of an acidified sample, formaldehyde being used as the binding agent in the blank determination as described by Ponting and Johnson[7]; for the sucrose - ascorbic acid mixture, this method gave poor end-points and Downer's method[8] was used. The results are shown in Table I.

Table I

COMPARISON OF RESULTS BY THE DESORPTION AND TRAPPING METHOD AND DIRECT IODIMETRY

Sugar solution	Total sulphur dioxide content, p.p.m.	Desorption and trapping method: Free sulphur dioxide found, p.p.m.	Desorption and trapping method: Desorption flow rate, ml per minute	Desorption and trapping method: Time required for desorption, minutes	Free sulphur dioxide found by direct iodimetry, p.p.m.
Dextrose, 10 per cent. w/v in acetate buffer	240	128	250	30	132
Liquid glucose, 10 per cent. w/v in acetate buffer	245	191	300	20	180
Fructose, 10 per cent. w/v in acetate buffer	258	254	300	20	245
Sucrose, 10 per cent. w/v in acetate buffer	251	258	300	20	245
Sucrose, 10 per cent. w/v in acetate buffer containing 100 mg of ascorbic acid per 100 ml . .	213	213	1500	15	224

During these experiments it was observed that a small amount of sulphur dioxide dissolved in droplets of moisture on the side of the desorption tube, but losses were avoided by washing back once during the desorption period. The size of desorption tube and the desorption flow-rate were also considerably increased at this stage. Recoveries of sulphur dioxide from standard solutions of sodium metabisulphite prepared in aqueous glycerol (to prevent oxidation) were carried out by the refined method. Results are shown in Tables II, III and IV.

TABLE II

PROPORTION OF TRAPPED SULPHUR DIOXIDE IN FIRST TRAPPING TUBE

Total sulphur dioxide trapped, mg ..	3·53	4·42	5·25	6·09	7·58	8·31
Percentage in first tube	100	100	100	99	99	99

TABLE III

RECOVERY OF SULPHUR DIOXIDE FROM STANDARD SOLUTIONS (IODIMETRIC METHOD)

Sulphur dioxide added, mg ..	0·47	0·94	1·40	1·88	2·81	3·75	4·69	5·63	7·50	8·44	9·38
Sulphur dioxide recovered, mg	0·49	0·93	1·38	1·88	2·77	3·73	4·72	5·61	7·41	8·47	9·28

Average recovery, 100 per cent. Range, 99 to 104 per cent.

TABLE IV

RECOVERY OF SULPHUR DIOXIDE FROM STANDARD SOLUTIONS (COLORIMETRIC METHOD)

Sulphur dioxide added, mg ..	0·31	0·62	0·94	1·25	1·56	1·87	2·18
Sulphur dioxide recovered, mg	0·32	0·61	1·02	1·20	1·56	1·86	2·13

Average recovery, 100 per cent. Range, 96 to 108 per cent.

In the colorimetric method only one tenth of the volume of liquid in the traps is normally taken for measurement, but for extremely low concentrations of sulphur dioxide a larger volume can be taken to make the method more sensitive.

METHOD

PREPARATION OF APPARATUS—

Set up the apparatus as shown in Fig. 1. More than one assembly may be used by one operator. The flow-rate required is approximately 1½ litres per minute of oxygen-free nitrogen for each assembly. This is conveniently controlled by use of a gas regulator with built-in flowmeter (British Oxygen Gases Ltd., Medical Division, Oxygen Regulator No. 330018).

Lightly grease the interchangeable joints of the apparatus. Into each of the two trapping tubes place 10 ml of 10 per cent. v/v glycerol in 0·1 N potassium hydroxide (alkaline glycerol reagent) if the iodimetric method is to be used or 10 ml of sodium tetrachloromercurate reagent[6] if the colorimetric method is to be used. The iodimetric method is preferred for 1 to 10 mg and the colorimetric method for smaller amounts up to 2 mg of trapped sulphur dioxide. Place 25 ml of water or 10 per cent. v/v glycerol in water (as specified for the type of sample to be examined) in the desorption tube. Pass a stream of air-free nitrogen through the assembly at 1½ litres per minute for approximately 5 minutes, and check that the joints are gas-tight.

PREPARATION OF SAMPLE—

Samples containing not more than 50 *per cent. of solids or* 400 *p.p.m. of sulphur dioxide*—Use distilled water in the desorption tube when preparing the apparatus. After passing nitrogen for 2 to 3 minutes, fairly quickly carry out the sequence of operations described below. Weigh 25 ± 0·02 g of sample into a second desorption tube. Add a few drops of water-soluble thymol blue and sufficient 5 N sulphuric acid to change the colour of the indicator from orange-yellow to red, and add 1 drop in excess. (If the colour of the sample interferes, determine the amount of 5 N sulphuric acid required on a separate equivalent portion with thymol blue as an external indicator or by using a pH meter, and then add the same amount of acid to the portion under test.) Turn off the flow of nitrogen. Replace the desorption tube containing water with the one containing the acidified sample, and ensure that the joint is gas-tight. If the samples show a marked tendency to froth, place a smear of Midland Silicones Ltd. anti-foam paste A on the side of the desorption tube.

Samples containing more than 50 per cent. of solids or 400 p.p.m. of sulphur dioxide—Use 10 per cent. glycerol in the desorption tube when preparing the apparatus and, after passing nitrogen for approximately 5 minutes, fairly quickly carry out the sequence of operations described below. Remove the desorption tube, but do not empty it. Weigh by difference a suitable portion of sample, and add it to the desorbed 10 per cent. glycerol in the desorption tube, ensuring that none of the sample adheres to the side of the tube. (For samples containing up to 400 p.p.m. of free sulphur dioxide, 25 g is a suitable amount of sample to use; decrease the amount of sample taken as the expected content of free sulphur dioxide increases.) Stir to dissolve, and acidify in exactly the same way as described in the previous paragraph. Replace the desorption tube containing the dilute acidified sample, and ensure that the joint is gas tight.

DESORPTION OF FREE SULPHUR DIOXIDE—

Turn on the supply of nitrogen, adjust the rate of flow to approximately 1½ litres per minute, and desorb at room temperature for 10 minutes. Turn off the nitrogen, disconnect the assembly between the desorption tube and the first trapping tube, and wash down the outlet tube of the wash bottle head and the walls of the desorption tube with a jet of distilled water. Reconnect the apparatus, and desorb for a further 5 minutes. Turn off the nitrogen flow, and combine the contents of the trapping tubes, washing out the tubes with distilled water. Recharge the trapping tubes, desorb for a further 5 minutes, and combine the contents separately. (For most samples it will be possible to omit the second desorption period if the conditions during the first period are rigidly observed; the full procedure, however, must be carried out for the first few determinations in each product to ascertain if this is possible.)

MEASUREMENT OF SULPHUR DIOXIDE IN THE TRAPPED LIQUIDS—

Iodimetric method—To the combined alkaline glycerol solutions in a 150-ml conical flask add a few drops of thymol blue and sufficient 5 N sulphuric acid just to give a red colour. Titrate with 0·02 N iodine to the first permanent blue colour, with starch or starch substitute as indicator. Simultaneously carry out a reagent blank determination by acidifying and titrating 20 ml of alkaline glycerol in the same way.

$$\text{Free sulphur dioxide content, p.p.m. w/w} = \frac{32{,}000\,(t - b)\,\text{N}}{w}$$

where t and b are the titres (in millilitres) for the sample and blank solutions, respectively, N is the exact normality of the sodium hydroxide solution and w is the weight of sample taken (in grams).

Colorimetric method—Combine the trapped sodium tetrachloromercurate solutions in a 50-ml calibrated flask, wash in quantitatively, dilute to the mark with distilled water, and mix. Complete the determination on a suitable portion (usually 5 ml) by using the colour developing procedure described by Beetch and Oetzel.[6]

RESULTS

The method was applied to some soft drinks and a concentrate. Results compared with those obtained by Downer's method of direct iodimetry (with electrometric titration when the colour of the sample interfered) are shown in Table V.

It is considered that there is satisfactory agreement between the results for free sulphur dioxide obtained by the two different methods. Difficulty was found in obtaining reliable results by direct iodimetry for the glucose beverage because of its low sulphur dioxide content and for the compounded blackcurrant juice because of its colour and complex nature. This probably accounts for the apparent disparity between the results for these samples.

In all the desorption and trapping determinations complete desorption occurred generally within 15 minutes and always within 20 minutes. No subsequent dissociation of free sulphur dioxide of any significance was observed. The method has since been applied to carbonated lemon drinks containing free sulphur dioxide in the range 3 to 40 p.p.m. and has given precise results after 15 minutes desorption; further desorption did not yield any more free sulphur dioxide.

TABLE V

COMPARISON OF RESULTS BY DIFFERENT METHODS

Sample	Total sulphur dioxide, p.p.m.	Free sulphur dioxide found by—	
		desorption and trapping method, p.p.m.	direct iodimetry, p.p.m.
Lemon squash	300	169, 161 (I)	167, 166
Orange squash	269	156, 156 (I)	152, 154
Lemon barley A	345	232, 240 (I)	232, 236
Lemon barley B	237	83, 83 (I)	62, 62
Lemon barley C	225	70, 73 (I)	66, 62
Blackcurrant cordial	311	178, 180 (I)	179, 177
Lemon juice	317	132, 135 (I)	132, 122
Liquid glucose beverage	30	11, 11 (C)	5
Lime juice cordial A	295	193, 191 (I)	196, 197
Lime juice cordial B	237	116, 116, 112 (I)	116, 114
Lime juice cordial B with added dehydroascorbic acid	225	48, 49 (I)	47, 43
Syrup of blackcurrant	301	99, 112 (I)	97, 120
Concentrated blackcurrant juice compound	1000 to 1200	160 (I)	203

I = Iodimetric method. C = Colorimetric method.

CONCLUSIONS

The work described has shown that, for a variety of soft drinks and raw materials, desorption and trapping provides a valid method of determining free sulphur dioxide.

The desorption and trapping method takes rather longer than do the iodimetric methods, but, by using two or more assemblies, one operator can complete at least four determinations in an hour. The apparatus required is relatively simple.

Advantages of the proposed method over direct iodimetric methods are: low or negligible blank values; elimination of drifting end-points and hence factors of personal judgement; application to samples of high and low solids content, high and low free sulphur dioxide content and to coloured and colourless samples.

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The Flame Photometric Determination of Sodium, Potassium and Calcium in Nickel-Alumina Catalysts

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A method is described for determining sodium, potassium and calcium in the range 0·05 to 4 per cent. in the presence of a large excess of nickel and alumina. The nickel and alumina are removed, as oxinates, by solvent extraction; the sodium, potassium and calcium are then determined with an E.E.L. flame photometer.

THE determination of sodium, potassium and calcium in nickel - alumina catalysts is of interest as sodium and potassium may be added as promoters and sodium and calcium may be introduced into the catalyst during its manufacture, *e.g.*, during washing. The composition of the catalysts vary, but they may contain up to 50 per cent. of nickel and up to 75 per cent. of alumina. Sodium, potassium and calcium in low concentrations are usually determined by flame photometry, the method being rapid and accurate provided interfering elements are removed. Nickel and alumina are present in large excess in the catalysts being considered; Pinta and Bove,[1] Ettre and Ádám,[2] Schuhknecht and Schinkel[3] and others have shown that they interfere with the flame emission produced by sodium, potassium and calcium. They must therefore be removed if flame photometry is to be used to determine these elements.

It seemed feasible to remove nickel and aluminium as oxinates because they both form complexes soluble in chloroform. Calcium oxinate is insoluble in chloroform, and there is no evidence that sodium and potassium form oxinates. Extraction of an aqueous digest should therefore separate the elements in the manner required. It has been shown that the extraction of nickel is facilitated by adding tartrate,[4] but this has been found to affect the determination of calcium and potassium because of their precipitation as tartrates. Moeller[5] has shown that, if pH is controlled, nickel can be removed from the solution by successive extraction without the use of tartrate.

EXPERIMENTAL

FLAME EMISSIONS PRODUCED BY NICKEL AND ALUMINIUM—

The flame emissions of nickel and aluminium were determined with an E.E.L. flame photometer (Evans Electroselenium Ltd.) on solutions prepared from analytical-reagent grade nickel nitrate and laboratory-reagent grade aluminium nitrate, the appropriate filters for sodium, potassium and calcium being used. The solutions contained nickel and aluminium in the same concentrations as would be present in typical acid digests of catalysts. Hydrochloric acid was added in an amount sufficient to give an acid concentration the same as that used to dissolve catalysts. The reagents tested were typical of those used in the

TABLE I

FLAME EMISSIONS PRODUCED BY SYNTHETIC CATALYST SOLUTIONS

Composition of catalyst in the solid state	Sodium		Potassium		Calcium	
	Flame photometer reading	Oxide concentration, p.p.m.	Flame photometer reading	Oxide concentration, p.p.m.	Flame photometer reading	Oxide concentration, p.p.m.
Nickel, 10 per cent. Alumina, 75 per cent.	6½	0·6	3	0·3	9½	9·2
Nickel, 25 per cent. Alumina, 50 per cent.	20	1·8	18½	1·8	49	25
Nickel, 50 per cent. Alumina, 25 per cent.	29½	2·7	53	5·2	>100	>50

preparation of catalysts. The flame photometer was set so that full-scale deflection (100 divisions on the galvanometer) was equivalent to 10 p.p.m. of sodium oxide, 10 p.p.m. of potassium oxide and 50 p.p.m. of calcium oxide. The galvanometer deflections and metal-oxide equivalents for three synthetic catalyst digests are shown in Table I.

The results show either that the reagents used already contain sodium, potassium and calcium, the latter in relatively high concentration, or that the nickel and aluminium have a natural flame emission at the wavelengths normally used for determining sodium, potassium and calcium. In the latter instance, the emission masks the traces of sodium, potassium and calcium, which must be assumed to be present, and makes it impossible to determine sodium, potassium and calcium by flame photometry. It has been shown that the emission was due to nickel and aluminium and not to relatively high concentrations of sodium, potassium and calcium by determining in a true blank solution the traces of sodium, potassium and calcium remaining after the nickel and aluminium had been extracted from portions of the same solutions.

DETERMINATION OF TRUE BLANK VALUES—

The synthetic catalyst digests used for determining the flame emissions produced by nickel and aluminium were extracted by the technique described below. Ten-millilitre portions of the solutions were extracted with 25 ml of 5 per cent. w/v 8-hydroxyquinoline (oxine) solution in chloroform; it was observed that very little nickel was extracted. When ammonium hydroxide was added and the solution was shaken, the nickel readily passed into the organic phase as the dark-green nickel oxinate. Addition of more ammonium hydroxide caused aluminium oxinate to be formed as a yellow precipitate that could only be re-dissolved with difficulty when the sample was extracted with pure chlorofom. Alternate extraction with a 5 per cent. solution of oxine in chloroform and then with pure chloroform, together with a gradual increase in pH (by adding ammonium hydroxide, sp.gr. 0·88) to 8 to 9, gave a satisfactory extraction procedure. The metals were found to be completely removed, and the gradual change in pH minimised separation difficulties due to the production of a flocculent aluminium oxinate precipitate. The aqueous solution after extraction was no longer green, but had a brown tint, presumably due to a small amount of dissolved oxine. A final washing with ethyl acetate made the aqueous phase the lower layer, which facilitated a quantitative transfer from the separating funnel. The aqueous solution was boiled to remove all dissolved organic solvents, as these could cause enhanced results[6,7] in the subsequent flame photometry. The solution was made up to 50 ml in a calibrated flask, and used directly in the E.E.L. flame photometer, which was set so that a full-scale deflection was equivalent to 10 p.p.m. of sodium oxide, 10 p.p.m. of potassium oxide and 50 p.p.m. of calcium oxide. A blank determination was made on 25 ml of distilled water by taking it through the same extraction procedure. The results are shown in Table II.

TABLE II

FLAME PHOTOMETER READINGS FOR DISTILLED WATER AND SYNTHETIC CATALYST SOLUTIONS AFTER EXTRACTION WITH OXINE

A flame photometer reading of 100 is equivalent to 10 p.p.m. of sodium oxide
10 p.p.m. of potassium oxide and 50 p.p.m. of calcium oxide

Element	Distilled water	Digest from synthetic catalyst—		
		A*	B†	C‡
Sodium	7, 9	15	16	15
	9, 8	15·5	15	15·5
	9, 9	15	15	15·5
Potassium	2, 2·5	3	2·5	3
	2, 2	3	3	3
	2·5, 2·5	2	2·5	2·5
Calcium	<0·5	<0·5	<0·5	<0·5

* Contains 10 per cent. of nickel and 75 per cent. of alumina.
† Contains 25 per cent. of nickel and 50 per cent. of alumina.
‡ Contains 50 per cent. of nickel and 25 per cent. of alumina.

The results in Table II show that the nickel - aluminium concentration has been reduced to a level that did not cause interference in the determination of sodium, potassium and calcium, and also that very little sodium, potassium and calcium are introduced by the reagents used in the extraction procedure. The concentrations of potassium and calcium in both the distilled water and the synthetic catalyst solutions are virtually identical, indicating complete removal of the interference due to nickel and aluminium. The slightly increased level of sodium in the synthetic catalyst solutions is well within the maximum permissible level of impurities in the reagents used for the preparation of the solution.

DETERMINATION OF RECOVERY LEVELS—

Five-millilitre portions of a solution containing 100 p.p.m. of potassium oxide and 100 p.p.m. of sodium oxide were transferred by pipette to 100-ml beakers together with 5 ml of a solution containing 500 p.p.m. of calcium oxide; 10 ml of 10 per cent. v/v hydrochloric acid were added, and the solutions were boiled for 10 minutes, which was the average time normally required for a sample of catalyst to dissolve. The solutions were set aside to cool, and then transferred to 100-ml separating funnels and taken through the extraction procedure described. Subsequently, the solutions were made up to 50 ml in calibrated flasks and used directly for determining potassium and calcium. Sodium was determined on a portion diluted with an equal volume of water. The results obtained were corrected for blank values, and the percentage recoveries were calculated. The results are shown in Table III.

TABLE III

RECOVERY VALUES FOR SODIUM, POTASSIUM AND CALCIUM

Sodium (5 p.p.m. present)			Potassium (10 p.p.m. present)			Calcium (50 p.p.m. present)		
Flame photometer reading	Sodium oxide equivalent to reading, p.p.m.	Average corrected for blank value, p.p.m.	Flame photometer reading	Potassium oxide equivalent to reading, p.p.m.	Average corrected for blank value, p.p.m.	Flame photometer reading	Calcium oxide equivalent to reading, p.p.m.	Average corrected for blank value, p.p.m.
60	5·34	5·10* (Recovery, 102%)	86	8·42	8·45 (Recovery, 84·5%)	98·5	49·2	49·2 (Recovery, 98·4%)
61	5·43		85	8·27		98·0	49·0	
61	5·43		86	8·42		98·5	49·3	
62	5·55		87	8·51		99·0	49·5	
60	5·34		86	8·42		98·0	49·0	
61	5·43		88	8·65		98·5	49·2	
			86	8·42				
			85	8·27				
			86	8·42				

* The solution was diluted (1 + 1), therefore the blank value was equivalent to 0·33 p.p.m. of sodium oxide.

The results show that the recovery for calcium and sodium is effectively quantitative, when the number of operations involved in the method is taken into consideration. The slightly high recoveries for sodium may be due to some sodium being extracted from the beakers during the digestion with boiling hydrochloric acid. The recovery for potassium was not quantitative, but the percentage was sufficiently constant to make acceptable the use of a correction factor in subsequent determinations. The loss of potassium may be caused by its slight solubility in organic solvents.

These preliminary trials show that nickel and aluminium can be removed from the digests of catalysts to give a solution in which the sodium, potassium and calcium can be directly determined with a flame photometer. A factor is however required in the determination of potassium.

METHOD

REAGENTS—

Ammonium hydroxide, sp.gr. 0·88—Analytical-reagent grade.

Hydrochloric acid, 10 *per cent. v/v*—Analytical-reagent grade.

8-*Hydroxyquinoline* (*oxine*) *solution,* 5 *per cent. w/v*—Dissolve analytical-reagent grade oxine in chloroform.

Chloroform.

Ethyl acetate.

Standard sodium - potassium stock solution—Dissolve 0·1886 g of analytical-reagent grade sodium chloride in distilled water. Dissolve 0·1583 g of analytical-reagent grade potassium chloride in distilled water. Combine the solutions, dilute to 1 litre, and store in a plastic bottle. The solution contains 100 p.p.m. of sodium oxide and 100 p.p.m. of potassium oxide.

Standard sodium - potassium working solution—Dilute the stock solution 1 + 9. Prepare freshly each week, and store in a plastic bottle. The solution contains 10 p.p.m. of sodium oxide and 10 p.p.m. of potassium oxide.

Standard calcium stock solution—Dissolve 1·785 g of analytical-reagent grade calcium carbonate in 100 ml of 4 per cent. hydrochloric acid, boil the solution to expel carbon dioxide, cool, and dilute to 1 litre. The solution contains 1000 p.p.m. of calcium oxide.

Standard calcium working solution—Dilute the stock solution 1 + 19. The solution contains 50 p.p.m. of calcium oxide.

PROCEDURE—

Weigh accurately about 0·25 g of catalyst in a 100-ml Pyrex-glass beaker, add 10 ml of 10 per cent. hydrochloric acid, cover the beaker with a watch glass, gently boil until a clear solution is obtained, cool, and quantitatively transfer the solution to a 100-ml separating funnel. Add 25 ml of oxine solution, shake, add 10 drops of ammonium hydroxide, sp.gr. 0·88, shake for approximately 3 minutes, allow the phases to separate, and run off and discard the lower (chloroform) phase. Add 5 drops of ammonium hydroxide, sp.gr. 0·88, and 25 ml of chloroform, shake for approximately 3 minutes, allow the phases to separate, and discard the lower phase. Add 25 ml of oxine solution, shake, add 5 drops of ammonium hydroxide, sp.gr. 0·88, shake, adjust the pH of the aqueous phase to 8 to 9 (2 to 3 drops of ammonium hydroxide are usually required), shake for 3 minutes, allow the phases to separate, and discard the lower phase. Wash the aqueous phase twice with 25-ml portions of chloroform, and discard the lower layers. Add 25 ml of ethyl acetate, shake for approximately 3 minutes, allow the phases to separate, and run off the lower phase into a 100-ml Pyrex-glass beaker. Wash the ethyl acetate layer twice with 5-ml portions of distilled water, adding the washings to the aqueous extract in the 100-ml beaker. Boil the extract until it is free from chloroform, cool, and make up to 50 ml in a calibrated flask. Carry out a blank determination by taking 10 ml of 10 per cent. hydrochloric acid through the proposed procedure. Determine the percentage recovery on 5 ml each of the stock standard sodium - potassium and calcium solutions with 10 ml of 10 per cent. hydrochloric acid by the proposed procedure. Dilute the samples (see Note 1), and dilute a portion of the "recovery" solution (1 + 1) with distilled water. Determine the sodium, potassium and calcium with a flame photometer; use the undiluted "recovery" solution for potassium and the diluted solution for the sodium and calcium. Correct the results for recovery of standards and reagent blank values.

NOTES—

1. The dilution factors, when 0·25 g of catalyst and a final volume of 50 ml were used, were—

0 to 0·2 per cent. of sodium or potassium oxide—no dilution.
0·2 to 2·0 per cent. of sodium or potassium oxide—dilute 1 + 9.
2·0 to 4·0 per cent. of sodium or potassium oxide—dilute 1 + 19.
No dilution is usually required for calcium.

2. For the E.E.L. flame photometer with standard E.E.L. filters, the full-scale deflection is set to be equivalent to 10 p.p.m. of sodium oxide, 10 p.p.m. of potassium oxide and 50 p.p.m. of calcium oxide. A graph plotted of galvanometer deflection against concentration of sodium and potassium was curved and that for calcium was a straight line.

RESULTS

Four catalysts (50 per cent. of nickel, 25 per cent. of alumina) have been analysed in quadruplicate for sodium, potassium and calcium; the results are shown in Table IV.

TABLE IV

QUADRUPLICATE ANALYSES OF 4 NICKEL - ALUMINA CATALYSTS

Results corrected for the recovery of standards and reagent blank values

Catalyst No.*	Sodium oxide found, %	Potassium oxide found, %	Calcium oxide found, %
I	All <0·01	1·34, 1·34, 1·38, 1·35	0·06, 0·08, 0·07, 0·07
II	0·08, 0·08, 0·08, 0·08	1·35, 1·33, 1·33, 1·33	0·15, 0·07, 0·13, 0·06
III	2·63, 2·60, 2·63, 2·67	4·56, —, 4·56, 4·54	0·17, 0·15, 0·14, 0·15
IV	0·05, 0·05, 0·05, 0·06	All <0·01	0·04, 0·01, 0·01, 0·01

* Catalyst No. I contained 1·0 per cent. of potassium oxide and a trace of sodium and calcium oxides.

Catalyst No. II contained 1·0 per cent. of potassium oxide, 0·1 per cent. of sodium oxide and a trace of calcium oxide.

Catalyst No. III contained 2·0 per cent. of potassium oxide, 0·1 per cent. of sodium oxide and a trace of calcium oxide.

Catalyst No. IV contained 0·1 per cent. of sodium oxide and a trace of potassium and calcium oxides.

The results were reproducible for a given catalyst and, with the exception of catalyst No. III, they agreed with the known approximate composition.

As a check on (1) the validity of the results for catalyst No. III and (2) the quantitative nature of the method, a known amount of sodium, potassium and calcium was added to known weights of samples of catalyst Nos. III and IV; these samples were then extracted and determined by the proposed procedure. On the assumption that the results in Table IV are correct, the average recoveries were 95 per cent. of sodium, 98·5 per cent. of potassium and 94 per cent. of calcium. This confirmed that quantitative results were being obtained and that the analysis of catalyst No. III was correct. The wide spread of the relatively small amount of calcium in the sample of catalyst No. II may be due to inhomogeneity of the sample.

I thank the Gas Council for permission to publish this paper.

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Received *October 9th*, 1962

SHORT PAPERS

Determination of Fluoride by Complexometric Titration

By M. A. LEONARD

(*Boots Pure Drug Co. Ltd., Analytical Development Group, Standards Department, Station Street, Nottingham*)

THE direct titration of fluoride ions in aqueous solution with solutions of metal salts, such as thorium nitrate, suffers from lack of stoicheiometry and from adsorption of the indicator on the gelatinous precipitate. Precipitation of fluoride with excess of such reagents as calcium chloride or cerous nitrate, with titration of the excess of metal ion in the filtrate, frequently gives rise to precipitates of an intractable gelatinous nature or of dubious composition.

Lead chlorofluoride can, however, be precipitated quantitatively from a suitable solution in a coarse crystalline form of definite composition over a wide range of conditions. Also, in conjunction with this, lead ions are easily and accurately titrated with ethylenediaminetetra-acetic acid (EDTA).

In the proposed method fluoride is precipitated, in the presence of chloride and ethanol, with standard 0·05 M lead nitrate, a reagent now available in bulk in many routine laboratories.

Laszlovsky first introduced the general principle mentioned above, but titrated the lead content of the precipitate. Titration of the excess of precipitant in the filtrate is usually a simpler and more rapid procedure and only becomes impracticable when a great excess of precipitant is needed.

Vřešťál and co-workers[1] precipitated fluoride with 150-ml portions of a saturated solution of lead chloride. This did not appear to be a particularly suitable standard reagent for routine use. Their titration of lead in the filtrate with EDTA, with xylenol orange as indicator, is similar to the proposed method.

Sakharova and Shishkina[2] precipitated lead chlorofluoride from dilute acetic acid with standard lead acetate solution; I regard the presence of acetate ions as detrimental to such a precipitation (see Table I). They continued by titrating lead ions in the filtrate with EDTA at pH 10 to 11 and used tartrate as secondary complexing agent and Solochrome black T as indicator.

METHOD

PROCEDURE—

Dissolve 5 to 45 mg of dry AnalaR or "Extra Pure" sodium fluoride in 20 ml of water, and add 8·0 ml of 0·2 M sodium chloride and 13 ml of 95 per cent. ethanol. Heat almost to boiling, and add from a burette 25·00 ml of standard 0·05 M lead nitrate, dropwise at first, then more rapidly as the precipitate becomes established. Swirl the solution constantly. Maintain the mixture just at the boiling-point for 1 minute to coagulate the precipitate, cool to room temperature, filter, and wash the residue with four 15-ml portions of 20 per cent. v/v ethanol in aqueous 0·02 M sodium chloride. Titrate the filtrate and washings with standard 0·05 M EDTA with xylenol orange as indicator and solid hexamine to bring the pH to approximately 6.

1 ml of 0·0500 M lead nitrate ≡ 2·10 mg of sodium fluoride.

RESULTS AND DISCUSSION

Sixteen concurrent determinations carried out on 30- to 45-mg samples of pure sodium fluoride gave a mean recovery of 100·0 per cent. with a standard deviation of 0·19 per cent.

In the absence of fluoride, lead chloride does not precipitate unless the alcohol content exceeds 43 per cent. v/v. This does not rule out possible co-precipitation, though the relation between recovery and volume of solution at the precipitation stage indicates that there is little cancelling of errors due to co-precipitation and precipitate solubility. Some results showing the variation in recovery of 42 mg of sodium fluoride with the volume at precipitation, the concentrations of ethanol and sodium chloride being maintained at the levels indicated under "Method," were—

Total volume, ml	100	200	250
Recovery, %	100·0	100·0	99·7

The choice of wash liquid is important as lead chlorofluoride is appreciably soluble in cold water. A 20 per cent. v/v solution of ethanol was reasonably effective, but addition of sodium chloride to 0·02 M introduced a common-ion effect that completely overcame this difficulty.

The concentration of ethanol present at the precipitation stage is not critical, but for the best results it should lie between 10 and 20 per cent., as indicated by the results shown below—

Concentration of ethanol, % ..	0	10·0	14·3	18·6	28·6	51·5
Recovery, %	98·7	99·6	100·0	100·0	100·6	101·6

The quality of the precipitate is inversely proportional to the concentration of ethanol. Ethanol may be replaced by acetone or methanol.

The ratio of chloride to fluoride (tested up to 4 to 1) is not critical once the 1 to 1 ratio has been exceeded. In the absence of chloride, lead fluoride precipitates to give 87 per cent. recovery based on lead fluoride. This recovery rises to 99·3 per cent. when the ratio of $[Cl^-]$ to $[F^-]$ reaches 1·1 to 1.

The excess of lead nitrate is not critical provided that the weight of chloride present is kept constant. If 25 ml of 0·05 M lead nitrate are taken for a final volume of approximately 70 ml, as in the method, then good recoveries can be obtained on sample weights of sodium fluoride from 4 to 46 mg.

For a total volume at precipitation of 70 ml, the temperature at filtration is not critical and may rise to as high as 50° C with negligible loss in recovery. However, at greater volumes this influence becomes more important, and temperatures should not exceed 25° C.

The pH of the fluoride solution before precipitation may be from at least 4·5 to 10·1, as indicated by the results shown below (pH was adjusted with dilute nitric acid or sodium hydroxide solution)—

pH	2·48	2·96	3·91	4·50	5·13	6·95	7·90	9·14	10·1	11·0
Recovery, %	80·4	86·4	95·8	99·5	99·6	99·7	99·8	100·0	100·0	101·1

At low pH precipitation is slow and incomplete, but large crystals are formed. Co-precipitation of basic lead salts does not cause interference until pH 11 is reached.

In the absence of alcohol and additional acid or alkali—

pH before precipitation = 6·5
pH after precipitation = 4·0
(pH of 0·05 M lead nitrate = 4·3)

Thus if no buffering component is present the pH of the mixture after precipitation appears to be fixed by the acid nature of the lead nitrate. If buffering ions are present in similar concentration to the lead nitrate, the results shown above for the variation in recovery with pH would no longer be valid.

The possible introduction of a buffer at pH 4 to 6 cannot be recommended, since this must contain a weak acid radicle that will complex to a greater or lesser extent with lead. This statement is amply borne out for acetate. Different volumes of an acetic acid - sodium acetate solution 0·50 M in acetate and of pH 5·0 were introduced into the method and the recoveries noted (see Table I); the concentration of chloride was kept constant.

TABLE I

INFLUENCE OF ACETATE

Volume of 0·5 M acetate added, ml	Ratio $\frac{[CH_3COO^-]}{[Pb^{2+}]}$	Recovery, %
2·0	0·8	98·7
5·0	2·0	96·9
10·0	4·0	93·0

The stability constant for the equilibrium—

$$Pb^{2+} + 2CH_3COO^- = Pb(CH_3COO)_2$$

has a value[3] of log K = 4·2, which would well explain this interference from acetate.

From the foregoing considerations it is evident that an unknown fluoride solution, which may contain buffering components, should be brought to a pH of 5 to 6 by addition of dilute nitric acid or sodium hydroxide only.

Interference in the method is caused by ions that precipitate or complex lead or fluoride; *e.g.*, sulphate and phosphate can be titrated almost quantitatively as fluoride.

The method was applied to solutions resulting from oxygen flask combustion of fluorinated organic compounds, but carbonate ions seriously interfered, and the time involved in removing them rendered the method no more rapid than some conventional colorimetric procedures.

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2. Sakharova, Ya. G., and Shishkina, N. I., *Zavod. Lab.*, 1959, **25**, 1442; *Anal. Abstr.*, 1960, **7**, 3239.
3. "Stability Constants of Metal-Ion Complexes, with Solubility Products of Inorganic Substances," Part 1, Special Publication No. 6, The Chemical Society, London.

Received *October 24th*, 1962

Book Reviews

LES CYCLITOLS: CHIMIE, BIOCHIMIE, BIOLOGIE. By THÉODORE POSTERNAK. Pp. 491. Paris: Hermann. 1962. Price 48 NF.

This book is devoted to those isocyclic poly-alcohols in which the hydroxyl groups are directly attached to the cyclic carbon atoms. Such compounds include the cyclohexane hexols, pentols, tetrols and triols, their alkyl ethers and cyclohexanone analogues (cycloses) occurring in various forms in nature and in synthetic varieties.

The treatise is exhaustive and authoritative, the author having devoted the greater part of his career to the subject. There are some 1280 references, but from the point of view of the analyst and particularly the analytical biochemist, the interest lies almost entirely in some 18 pages dealing with the determination of meso-inositol and its naturally occurring phosphoric esters, the most generally known of this group of compounds. Details of six chemical methods are described, but since chemical methods lack specificity and entail laborious isolation and purification procedures, microbiological procedures are those most frequently used. Six such methods and three enzymatic methods are given in detail.

The book is well produced, and the spatial formulae necessitated in order to clarify the reactions where so many stereo-isomers exist are a feature. J. I. M. JONES

ROCK-FORMING MINERALS. VOLUME 2. CHAIN SILICATES. W. A. DEER, M.Sc., Ph.D., F.G.S., R. A. HOWIE, M.A., Ph.D., F.G.S., and J. ZUSSMAN, M.A., Ph.D., F.Inst.P. Pp. xii + 379. London: Longmans, Green and Co. Ltd. 1963. Price 95s.

Thirty-six important minerals of the pyroxene and amphibole groups, together with wollastonite, pectolite, rhodonite, bustamite and pyroxmangite, are dealt with in a manner similar to that for the minerals described in other volumes of this work (see *Analyst*, 1962, **87**, 607 and 914; 1963, **88**, 246). L. S. THEOBALD

QUANTITATIVE CHEMICAL ANALYSIS AND INORGANIC PREPARATIONS. By R. M. CAVEN, D.Sc., F.R.I.C. Second Edition. Revised by A. B. CRAWFORD, B.Sc., Ph.D., A.R.C.S.T., F.R.I.C., and W. A. ALEXANDER, B.Sc., F.R.I.C. Pp. viii + 428. London and Glasgow: Blackie & Son Ltd. 1962. Price 35s.

The first edition of this book appeared in 1923 and was reprinted 20 times—a tribute to its value as a text-book. This revised edition has been largely rewritten, and is praiseworthy for its attempt to give up-to-date explanations of many of the reactions used in analysis, and for its departure from the older style of presentation. One feels, however, that much duplication of material might have been avoided if the elementary parts had been omitted and the beginning student credited with enough intelligence and interest to be able to appreciate the fuller exposition given later in the text. The space so saved could have been used to enlarge on such topics as redox reaction mechanisms, which are left tantalisingly after a brief mention of their existence.

It is important for the future that analysis should be integrated with the rest of a teaching course. Analysis may be a tool for use by the other branches of chemistry, but it also is a branch in its own right and must make the utmost use of developments in physical, inorganic and organic chemistry.

An excellent feature of the book is its emphasis on the analysis of the compounds prepared by the student. This gives the student a determination of the purity of his product and a real sense of one of the main purposes of analysis—quality control. At the price, which is a modest one for text-books nowadays, the book is extremely good value.

R. A. CHALMERS

ANALYTICAL CHEMISTRY OF POLYMERS. Part II. ANALYSIS OF MOLECULAR STRUCTURE AND CHEMICAL GROUPS. Edited by GORDON M. KLINE. Pp. xvi + 619. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1962. Price 132s.

ANALYTICAL CHEMISTRY OF POLYMERS. Part III. IDENTIFICATION PROCEDURES AND CHEMICAL ANALYSIS. Edited by GORDON M. KLINE. Pp. xii + 566. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1962. Price 124s.

The ever-growing industrial importance of polymeric materials has stimulated intensive studies of their structure and their chemical and physical properties, and in consequence most of the analytical techniques of chemistry and physics, the term "analytical" being used in its widest sense, have been employed in this field.

These two books complete Volume 12 of a series of monographs on the chemistry, physics and technology of high polymers (Part 1 deals with the analysis of monomers and polymeric materials including plastics, resins, rubbers and fibres and was published in 1959).

Part 2 describes the analysis of molecular structure and chemical groups and contains 12 chapters, each of which deals with a particular analytical technique, *i.e.*, molecular weight and size (including discussions of fractionation, viscosity, light scattering, osmometry and ultracentrifugation), X-ray diffraction, optical methods (including refractive index, birefringence, dichroism, optical rotation and light transmission), differential thermal analysis, pyrolysis, mass spectrometry, ultraviolet and infrared spectrophotometry, fluorescence, chromatography, polarography and magnetic resonance spectroscopy (which includes NMR and ESR). In each chapter the basic principles, instrumentation and methods of using a technique are discussed with examples of typical applications to polymeric materials, and the whole text is supported with over 1600 references.

Part 3 deals with identification procedures and chemical analysis. The first chapter (100 pages) deals with systematic procedures for the qualitative analysis of a basic polymer present in a plastic formulation after removal of the other constituents, involving its burning characteristics, products of pyrolysis and various simple chemical and physical properties. The second chapter (119 pages) contains a comprehensive account of the simple chemical colour tests that can be used for the identification of polymers or their constituents. The remaining chapters deal with microscopy (89 pages), applications of radiochemical analysis (47 pages) and the determination of end-groups (15 pages). These chapters are supported by just under 1000 references, and in a separate section a further 145 references are given "to books and reviews of general interest and to articles which have been published recently." The latter are given in alphabetical order of authors and are not referred to in the index, so that one has to work hard to make use of them. The fact that around 100 of these references are dated 1959 to 1961 confirms one's impression that both volumes were probably drafted several years ago and little account of the most recent literature has been taken in the published version. This is a pity, since much interesting work has been missed. The last 146 pages are taken up with cumulative author and subject indexes covering Parts 1 to 3.

Where it has been necessary to discuss the basic principles of an analytical technique in these volumes, this has been done well and could be of considerable interest to analysts in other fields. It is not easy to judge from the text, however, the relative practical importance of the various techniques in use to-day, and a critical discussion of this would be welcome in the next edition; for example, no stress is laid on the value of ultraviolet or infrared spectrophotometry and the growing importance of gas chromatography (especially in conjunction with pyrolysis) compared with chemical identification procedures and the use of fluorescence and polarography, the latter particularly being given full discussion somewhat out of proportion to its practical value. However, despite this and the absence of reference to the most recent developments, the three parts of Volume 12 now available do comprise a most valuable survey of the literature up to about 1959, which will repay careful study and be of great practical value to newcomers to the field of polymer analysis. A. G. Jones

Symbols, Units and Nomenclature in Physics. Document U.I.P. 9. (S.U.N. 61–44.) Recommendations composed by the Commission for Symbols, International Union of Pure and Applied Physics. Pp. 23. Obtainable in bulk from the Secretariat, I.U.P.A.P., 3 Boulevard Pasteur, Paris 15, France, Price P.B. 50 (approx. $15.00) 100 copies.

This document, which replaces earlier reports from the Symbols, Units and Nomenclature Commission of I.U.P.A.P., has been published with financial support from the UNESCO.

The scope of the recommendations is defined by the following list of sections included in the present edition—

1. Physical quantities—General recommendations.
2. Units—General recommendations.
3. Numbers and figures.
4. Symbols for chemical elements, nuclides and particles.
5. Quantum states.
6. Nomenclature.
7. Recommended systems for physical quantities.
8. Recommended mathematical symbols.
9. International symbols for units.

Appendix. Systems of quantities and units in electricity and magnetism.

The explanatory text is in English, but the names of the physical quantities, units and mathematical symbols in the classified lists are given in English and in French.

The recommendations in the report are in general agreement with recommendations of the following international organisations: International Organisation for Standardisation; General Conference on Weights and Measures; International Union of Pure and Applied Chemistry; International Electrochemical Commission; International Commission on Illumination.

Publications Received

Compound Semiconductors. Volume 1. Preparation of III-V Compounds. Edited by Robert K. Willardson and Harvey L. Goering. Pp. xxii + 553. New York: Reinhold Publishing Co.; London: Chapman & Hall Ltd. 1962. Price $25.

Some General Problems of Paper Chromatography: Relations Between Paper Chromatographic Behaviour and Chemical Structure: Attempts at Systematic Analysis. Edited by I. M. Hais and K. Macek. Pp. 220. Prague: Publishing House of the Czechoslovak Academy of Sciences. 1962. Price Kčs 22.50.

A Symposium organised by the Chromatography Group of the Czechoslovak Chemical Society at Liblice on 23rd June, 1961.

Bibliography of Paper Chromatography 1957–1960 and Survey of Applications. By Karel Macek, Ivo M. Hais, Jiří Gasparič, Jan Kopecký and Vlastimil Rábek. Pp. 706. Prague: Publishing House of the Czechoslovak Academy of Sciences. 1962. Price Kčs 87.

Technical Writing. By Richard W. Smith. Pp. x + 181. New York: Barnes & Noble Inc. 1963. Price $1.25 (paper); $3.50 (cloth).

Practical Methods for the Microbiological Assay of the Vitamin B-Complex and Amino Acids. By E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol. Pp. 52. London: United Trade Press Ltd. 1963. Price 10s. 6d.

Reprinted from Laboratory Practice.

The Case Against the Nuclear Atom. By Dewey B. Larson. Pp. x + 139. Oregon: North Pacific Publishers. 1963. Price $4.50.

Notice to Authors

THE Society publishes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Such papers may describe original work or may present in review form a critical evaluation of the existing state of knowledge on a particular facet of analytical chemistry. Papers may be submitted for publication by members of the Society or by non-members.

Papers and all correspondence relating thereto should be sent to the Editor of *The Analyst*, 14 Belgrave Square, London, S.W.1.

Every paper will be submitted to at least two referees, by whose advice the Editorial Committee of *The Analyst* will be guided as to its acceptance or rejection. Papers that are accepted must not be published elsewhere except by permission of the Committee. Submission of a manuscript will be regarded as an undertaking that the same material is not being considered for publication by another journal.

Manuscript—Papers should be typewritten in double spacing on one side *only* of the paper. Three copies (top and two carbon copies) should be sent to the Editor, and a further copy retained by the author.

Title and synopsis—The title should be brief but descriptive, and must pin-point the original features of the work. All papers must be accompanied by a short synopsis of about 100 to 250 words; this should give the principle of the method, draw attention to its novel features and indicate its scope and sensitivity. Contributions to the Short Papers section do not require synopses.

Proofs—The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned within 48 hours of receipt.

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NOTES ON THE WRITING OF PAPERS FOR *The Analyst*

Manuscripts should be in accordance with the style and usages shown in recent copies of *The Analyst*.* Conciseness of expression should be aimed at: clarity is increased by adopting a logical order of presentation, with suitable paragraph or section headings.

Descriptions of new methods should be supported by experimental results showing accuracy, precision and selectivity.

The recommended order of presentation is as indicated below—

(*a*) Synopsis.
(*b*) Statement of object of investigation and, if necessary, historical introduction and account of preliminary experimental work; these need be no longer than is necessary for the understanding of the new material.
(*c*) Description of method. When working details are given, they should, if possible, be given in the imperative mood. Well known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Statistical analysis of results. Any statistical evaluation of results should be in accordance with accepted practice.
(*f*) Discussion of scope and validity.
(*g*) Summary and conclusions.

Tables, diagrams, etc.—The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns may often be arranged horizontally. No lines should be ruled in tables in the manuscript. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

* Rules for nomenclature in "Handbook for Chemical Society Authors 1961" (price 21s. from The Chemical Society, Burlington House, London, W.1) are followed. The Shorter Oxford English Dictionary is followed for spelling, but some words are given that Dictionary's secondary alternative spelling.

Tables or graphs may be used, but not both for the same set of results, unless important additional information is given by so doing.

In general, graphs should have a reasonable number of co-ordinate lines, and not only the two main axes. The information given by a straight-line calibration graph can usually be conveyed adequately as an equation in the text.

Diagrams and graphs should be drawn in Indian ink on Bristol board, stout paper or tracing cloth, not larger than foolscap size and with at least 1-inch margins all round. The use of squared paper should be avoided. All lettering should be inserted lightly in black lead pencil at the appropriate place in the diagram, and will be replaced by type in block-making. All lines in Indian ink should be firmly drawn and sufficiently thick to stand reduction.

Drawings should be specially prepared for submission to *The Analyst*, as they cannot normally be returned and may be modified or cut in the course of block-making.

Three sets of illustrations should be provided, two sets of which may be photographic or dyeline copies of the originals, or pencil sketches, for transmission to the referee; there is no need to prepare Indian-ink duplicates.

Photographs—Photographs should only be submitted if they convey essential information that cannot be shown in any other way. They should be submitted as glossy prints made to give the maximum detail. Colour photographs can only be accepted when a black-and-white photograph fails to show some vital feature.

Abbreviations—Normality and molarity are generally expressed as decimal fractions (*e.g.*, 0·02 N, 0·375 M). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and after °C, °F, μ, Å and other units represented by symbols; litre and metre, when without prefixes, are printed in full.

Abbreviations other than those of recognised units should be avoided in the text; symbols and formulae are not used instead of the names of elements and compounds in the text, but may be used in addition to names when they are necessary to avoid ambiguity, *e.g.*, to specify crystalline composition, as in $CuSO_4.5H_2O$, to show structure or in equations.

Percentage concentrations of solutions should be stated as "per cent. w/w" (alternatively "g per 100 g"), as "per cent. w/v" (alternatively "g per 100 ml") or as "per cent. v/v." Concentrations of solutions of the common acids, however, are often conveniently given as dilutions of the concentrated acids, such as "diluted hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might be equivalent to *either* 1 + 4 *or* 1 + 3.

References—References should be numbered serially in the text by means of superscript figures, *e.g.*, Mackenzie and Mitchell[1] or Furman,[2] and collected in numerical order under "REFERENCES" at the end of paper. They should be listed, with the authors' initials, in the following form

1. Mackenzie, R. C., and Mitchell, B. D., *Analyst*, 1962, **87**, 420.
2. Furman, N. H., *Editor*, "Standard Methods of Chemical Analysis," Sixth Edition, D. Van Nostrand Co. Inc., New York and London, 1962, Volume 1, p. 863.

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the volume or page number, or both if required.

The entry of "personal communications" in the reference list is not justified; full acknowledgment of such unpublished sources should be made in the text or in the acknowledgments at the end of the paper.

Authors must, in their own interest, check their lists of references against the original papers; second-hand references are a frequent source of error. The number of references must be kept to a minimum.

JUNE, 1963 Vol. 88 No. 1047

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

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DEATHS

WE record with regret the deaths of

Frank William Greaves
Louis Francis McCallum
Alec Duncan Mitchell.

NORTH OF ENGLAND SECTION AND BIOLOGICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Biological Methods Group was held at 6 p.m. on Friday, April 5th, 1963, at the Lecture Theatre, Evans Medical Research Laboratories, Speke, Liverpool 24. The Chair was taken by the Chairman of the North of England Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

A lecture on "Pharmacological Studies of Habituation" was given by E. M. Glaser, M.C., Ph.D., M.D., M.R.C.P.

The meeting was preceded at 2 p.m. by a tour of the Evans Medical Research Laboratories.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Stirlingshire and District Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Friday, March 29th, 1963, at the Lea Park Tea-room, Callendar Road, Falkirk. The Chair was taken by the Chairman of the Scottish Section, Dr. R. A. Chalmers, B.Sc.

The following paper was presented and discussed: "The Forensic Chemist," by F. G. Tryhorn, D.Sc., F.R.I.C.

WESTERN SECTION

A JOINT Meeting of the Western Section and the South Wales Section of the Royal Institute of Chemistry was held at 6 p.m. on Friday, March 15th, 1963, in the New Science Building, Swansea College of Technology, Swansea. The Chair was taken by the Chairman of the South Wales Section, Mr. H. Evans, B.Sc., F.R.I.C.

A lecture on "Atmospheric Pollution" was given by B. T. Commins, M.Sc., Ph.D., A.R.I.C.

WESTERN SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the Western Section and the Physical Methods Group was held at 6.30 p.m. on Tuesday, March 26th, 1963, in the Department of Inorganic and Physical Chemistry, The University, Bristol. The Chair was taken by the Chairman of the Western Section, Dr. F. H. Pollard.

The subject of the meeting was "Determination of Pesticide Residues" and the following papers were presented and discussed: Introductory Talk by D. T. Lewis, C.B., Ph.D., D.Sc., M.R.S.H., F.R.I.C.; "Ordinate Scale Expansion Techniques in the Infrared Analysis of Pesticide Residues," by Miss G. P. Mansfield, B.Sc., and D. F. Muggleton, B.Sc., Ph.D., A.R.I.C.; "Mercury Residues in Plants," by J. A. Pickard and J. T. Martin, Ph.D., D.Sc., F.R.I.C.; "The Detection and Determination of Some Chlorinated Pesticide Residues in Crops, Soils and Animal Tissues by Gas - Liquid Chromatography," by R. Goulden, F.R.I.C. (see summaries below).

The meeting was preceded at 2.30 p.m. by a visit to Long Ashton Research Station.

ORDINATE SCALE EXPANSION TECHNIQUES IN THE INFRARED ANALYSIS OF PESTICIDE RESIDUES

DR. D. F. MUGGLETON said that the study of residues on growing plant material and harvested crops was now an integral part of the development of new pesticides. In consequence, satisfactory routine analytical methods were required for many compounds that might be present in fruit and similar material to the extent of only a few parts per million.

Until quite recently infrared measurements were relatively insensitive and usually required several milligrams of sample. With pesticides, therefore, they were of value only for production control. Now, however, as the result of improvements in instruments and technique, infrared spectroscopy could be used satisfactorily for residue studies involving microgram amounts of a compound. Indeed, it had several advantages—

(1) Most compounds exhibited several absorption bands in the readily accessible infrared region from 2 to 16 μ. These were available for both qualitative and quantitative studies so that complicated analytical procedures were avoided.

(2) Because every compound had a characteristic infrared spectrum, metabolites could readily be detected.

(3) "Clean-up" requirements were no more rigorous than for other methods, and indeed might be less so.

(4) The same basic equipment and technique were available for many compounds so that instrumentation and technician training were simplified.

Unfortunately, samples containing a few micrograms of pesticide might give extremely weak absorption bands, whose accurate measurement by conventional methods was difficult. Spectral bands sufficiently intense for precise measurement were obtained if the amount of energy absorbed by the sample was increased by using long path-length cells or beam-condensing optics. A more flexible approach was offered by ordinate scale expansion, which gave easily measured spectral bands, but required no large absorption of energy by the sample. It merely involved adjusting the spectrophotometer to the most favourable operating conditions and then increasing electronically the recorder pen deflection for a given change in sample transmittance. Some modification of the standard double-beam spectrophotometer was required, but the introduction of two precision potentiometers, one linked to the optical wedge and the other to the pen carriage, permitted appropriate expansion factors to be introduced at will. Factors of 5 ×, 10 × and 20 × were convenient for many purposes. With the potassium bromide pressed-disc technique about 1 mg of phosphamidon, for example, was required for a full-scale spectrum, but absorption bands of comparable intensity were obtained with 50 μg and 20 × scale expansion. Thus 5 μg of this compound could be detected, and this represented less than 0·1 p.p.m. on 100 g of plant material, since, in general, recoveries were not quantitative.

Ordinate scale expansion had useful applications in residue analysis. First, it permitted very small amounts of pesticide residue (about 0·1 p.p.m.) to be qualitatively identified and quantitatively measured on reasonably sized samples (about 100 g) of plant material. It might therefore increase the sensitivity of an established method and allow a reduction to be made in sample size. Secondly, it could be employed with samples in the liquid or solid state. The potassium bromide pressed-disc technique was very convenient for samples containing completely unknown amounts of residue and a wide range of expensive cells is not required. Finally, useful measurements could be made on relatively weak absorption bands that occurred at convenient points in the spectrum.

Infrared spectroscopy was thus of considerable value for residue studies and the sensitivity attainable with ordinate scale expansion compared favourably with that of other techniques.

MERCURY RESIDUES IN PLANTS

DR. J. T. MARTIN said that inorganic mercury compounds were used for soil and root treatment and organic mercury compounds were used in seed dressings, orchard sprays and glasshouse aerosols for the control of fungal diseases. The uptake of mercury by roots, leaves and fruits after applications at commercial rates had been followed in relation to the efficiency of disease control and to possible risks to the consumers of treated crops. Analytical methods had been developed for the determination of mercury residues in plant material (*J. Sci. Food Agric.*, 1960, **11**, 374) and soil (to be published).

The roots of lettuce and dwarf bean plants accumulated mercury from nutrient solution containing phenylmercuric acetate, but little translocation occurred to the foliage. Root treatment with calomel or mercuric chloride of cauliflower seedlings before planting led to absorption by the roots but the curds were uncontaminated. Carrots grown in soil treated with mercuric chloride contained up to 0·05 p.p.m. and roots from calomel-treated soil showed 0·02 p.p.m. of mercury when seed was sown immediately after soil treatment; delay in seeding eliminated contamination. Lettuces, dwarf beans, carrots, potatoes and turnips from field experiments involving calomel and mercuric oxide soil treatments showed mercury residues not exceeding 0·03 p.p.m.

Apple leaves absorbed mercury, deposited as phenylmercuric acetate, within a few days. Mercury was found in young coffee and citrus lime leaves that emerged after spraying with phenylmercuric acetate, indicating translocation. Broad bean plants sprayed with phenylmercuric acetate at early flowering later showed 0·02 p.p.m. in the pods, 0·04 p.p.m. in the seeds and 0·07 p.p.m. of mercury in the roots. Applications of phenylmercuric acetate to the leaves of potato plants led to contamination of the tubers (0·15 p.p.m. of mercury in peel, 0·18 p.p.m. in flesh) and roots (1·2 p.p.m. of mercury).

Each application of phenylmercuric acetate deposited up to 10 p.p.m. of mercury on apple leaves. The surface deposits varied in different parts of a tree, and fell to 2 to 3 p.p.m. between successive treatments; mercury within the leaves rose steadily to 3 p.p.m. Apple fruits from commercially sprayed orchards contained 0·05 p.p.m. of mercury distributed between the peel, flesh and core. Five applications of phenylmercuric acetate, under experimental conditions, gave 0·24 p.p.m. on whole fruits; one-third of the mercury was located in the flesh. Mercury deposited on the surface of the fruits was largely held in the cuticle; much of the mercury in the flesh arose from translocation from the leaves. Mercury was detected in the fruitlets (0·4 p.p.m.) and young leaves (0·07 p.p.m.) of trees sprayed the previous year. The bark of trees treated for six consecutive years contained 4 p.p.m. of mercury.

Naturally-occurring mercury in soils varied between 0·05 and 0·012 p.p.m. Soil from beneath sprayed apple trees contained 0·2 p.p.m. (untreated 0·06 p.p.m.). Soils treated with inorganic mercurials showed up to 2 p.p.m. of mercury (untreated 0·05 p.p.m.).

The results indicated that while absorption of mercury by roots was followed by little translocation to aerial parts, the penetration of mercury into leaves led to movement throughout the plant. The edible portions of crops treated under commercial conditions with mercurial fungicides contained less than 0·1 p.p.m. of mercury.

THE DETECTION AND DETERMINATION OF SOME CHLORINATED PESTICIDE RESIDUES IN CROPS, SOILS AND ANIMAL TISSUES BY GAS - LIQUID CHROMATOGRAPHY

MR. R. GOULDEN outlined a rapid method for the identification and determination of traces of chlorinated pesticides in crops, soils and animal tissues by gas - liquid chromatography with an essentially non-polar column with electron capture ionisation detection. Only conventional G.L.C. equipment was required, and neither preliminary "clean-up" nor concentration of the extract solution of the pesticide was, in general, necessary. The procedure was usually applicable to chlorinated pesticide residues in concentrations down to 0·1 to 0·25 p.p.m., and a further increase in method sensitivity for a particular pesticide could generally be achieved by adjustment of sample volumes or operating conditions or by the introduction of an extract "clean-up" stage or by some combination of these changes. Interference present in extracts from grain samples could be resolved by the use of a polar G.L.C. column or removed by liquid - solid chromatography. The standard procedure devised required about 50 minutes for a single analysis and only 30 minutes for serial analyses.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Thursday, March 14th, 1963, at the Wolverhampton and Staffordshire College of Technology, Wulfruna Street, Wolverhampton. The Chair was taken by the Vice-Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Refractory Analysis," by H. Bennett, B.Sc., A.R.I.C.

A JOINT Meeting of the Midlands Section with the Lea Valley Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Wednesday, April 24th, 1963, at the College of Technology, Park Square, Luton. The Chair was taken by the Chairman of the Lea Valley Section, Dr. J. Haslam, F.R.I.C.

The following paper was presented and discussed: "The Examination of Questioned Documents," by Professor C. L. Wilson, Ph.D., D.Sc., F.R.I.C., F.I.C.I.

MICROCHEMISTRY GROUP

THE thirty-ninth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, March 20th, 1963, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. D. W. Wilson, M.Sc., F.R.I.C.

A discussion on "Microchemical Problems in Air Pollution" was opened by T. Nash, M.A., B.Sc., A.R.I.C.

BIOLOGICAL METHODS GROUP

THE Group held a Symposium on "The Pharmacological Screening of New Drugs" at 10 a.m. on Wednesday, March 13th, 1963, in the Friends Meeting House, Euston Road, London, W.C.1.

The Chairman of the morning session was Dr. F. Hartley, F.P.S., F.R.I.C., and the following papers were presented and discussed: Introduction by the Chairman; "Organisation of Pharmacological Screening Tests," by M. W. Parkes, B.Sc., Ph.D.; "Cardiovascular Drugs," by A. F. Green, B.A.; "Anticonvulsant Drugs," by C. Cashin, B.Pharm., M.P.S.

The Chairman of the afternoon session was Dr. L. F. Wiggins, F.R.I.C., and the following papers were presented and discussed: "Analgesic Drugs," by A. Macfarlane, B.Sc.; "Anti-inflammatory Drugs," by S. S. Adams, B.Pharm., Ph.D., F.P.S.; "Anti-allergic Drugs," by W. G. Smith, B.Pharm., Ph.D., A.R.I.C., F.P.S.; Closing Remarks by the Chairman.

Analytical Methods Committee

REPORT PREPARED BY THE CHLORINE IN ORGANIC COMPOUNDS SUB-COMMITTEE

The Semi-micro-determination of Chlorine in Agricultural Technical Organic Chemicals and their Formulations

THE Analytical Methods Committee has received the following report from its Chlorine in Organic Compounds Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The work of the Sub-Committee resulted from a request from the Pesticides Analysis Advisory Committee of the Ministry of Agriculture, Fisheries and Food (PAC), who had been allocated by the Collaborative Pesticides Analytical Committee of Europe (CPAC) the task of preparing methods for determining compounds containing chlorine in organic combination in pesticides. Because PAC considered this problem to be one of general analytical chemistry rather than one specific to pesticides, the Analytical Methods Committee was asked to undertake this investigation.

A sub-committee was therefore set up under the chairmanship of Professor R. Belcher, and its membership was: Mr. J. H. Dunn, Mr. K. Gardner, Mr. R. Goulden, Mr. G. Ingram (who resigned during the course of the work) and Dr. A. M. G. Macdonald, with Dr. C. H. Tinker as Secretary; Mr. C. A. Johnson joined the Sub-Committee later. The Sub-Committee's terms of reference were "to prepare methods for the determination of organically bound chlorine, having special reference to commercial preparations such as pesticides."

INTRODUCTION

It was considered by the Sub-Committee that any method recommended should, if possible, be applicable to both volatile and non-volatile compounds. The work resolved itself into two parts: (*i*) the selection of a method for destroying the organic matter and bringing the chlorine into solution in a suitable form and (*ii*) the development of a method for determining the chlorine in the solution. Consideration was given to several methods for decomposing the sample, including the flask-combustion,[1] Stepanow,[2] peroxide-bomb[3] and combustion-train procedures.[4] A semi-micro method was considered desirable, because the results obtained with a micro method are likely to be adversely affected by heterogeneity of the sample, whereas a macro method was to be avoided, if possible, as the preliminary destruction of organic matter is likely to be less efficient with the large sample required. The ultimate choice was between the flask-combustion and Stepanow methods. The former was preferred rather than the latter, which, although easier to carry out in all laboratories because it does not require any special apparatus, is not suitable for all types of compounds, particularly pentachlorophenol. The flask-combustion method is easy to manipulate and more universally applicable, although during the work of the Sub-Committee it was shown to have limitations, borne out in the published chemical literature, particularly when the chlorine was present in the organic matter in low concentrations and when inorganic fillers largely predominated in formulations. The decision to use the flask-combustion method was justified by the collaborative trials, and the method finally recommended in the Appendix (see p. 417) is based on this technique. It should be noted that this method determines total organic and ionisable chlorine, and if ionisable chlorine is present in the material a correction must be made.

The determination of the hydrochloric acid in the solution resulting from the decomposition required investigation. Several methods are feasible. Potentiometric titration with standard silver nitrate solution was considered preferable, but not all laboratories have the necessary apparatus. The method involving the addition of mercuric oxycyanide to the neutralised solution and titration of the liberated alkali with standard acid is sensitive, but required study to establish the best method of determining the end-point. Direct alkalimetry proved applicable only in special instances. After completion of the collaborative work, several members of the Sub-Committee examined Cheng's method,[5] as modified by White,[6] which involves the use of a solution of mercuric nitrate for a direct titration in aqueous ethanol, and found it satisfactory in their hands.

EXPERIMENTAL

A considerable amount of experimental work was undertaken, as a result of which improvements were made to the technique originally proposed. The need for special precautions at certain stages was observed and difficulties that arose in the work by individual members of the Sub-Committee were noted. The method finally recommended has been drafted in the light of this experience. Points needing special emphasis are summarised below.

(*i*) Although a 500-ml flask is adequate for the decomposition of many materials likely to be encountered, a 1-litre flask is specified, as this will be suitable for all the materials for which the method is recommended, including miscible-oil products.

(*ii*) A cylindrical platinum-gauze sheath, rather than a spiral, is recommended as this provides a better catalytic action to assist combustion and also holds the sample more securely. The suggested dimensions of the combustion apparatus are such that the possibility of the flame impinging on a cold glass surface, causing soot formation accompanied by incomplete decomposition, is minimised.

(*iii*) An absorbent solution consisting of water and hydrogen peroxide is recommended in order to preclude the formation of any hypochlorite. Moreover, the peroxide facilitates removal of the carbon dioxide resulting from the combustion of certain materials; this carbon dioxide, if present, interferes with the oxycyanide method for determining the chloride in the absorbent solution.

(*iv*) The presence of small amounts of soot after combustion does not appear to affect results obtained with the potentiometric finish. Soot does, however, obscure the end-point in the oxycyanide finish, and its formation should therefore be avoided.

(*v*) With liquid materials, the sample should be weighed into a suitable hard capsule containing ashless filter-paper floc as an absorbent. Some types of hard gelatin capsules contain a significant amount of chloride, and before a particular batch of capsules is accepted as satisfactory it should be examined to establish that its chloride content is low. Methylcellulose capsules contribute little chloride to the blank and do not give rise to acidic combustion products that would invalidate an alkalimetric finish; they are therefore preferable.

(*vi*) The recommended method is suitable for pure organic chlorine-containing substances and for many formulations, provided that the chlorine content exceeds about 5 per cent. However, low results have been reported for one highly chlorinated material. For routine purposes, satisfactory results may be obtained with materials containing as little as about 1 per cent. of chlorine. Attempts to apply the method to formulations containing about 0·5 per cent. of chlorine in a largely inorganic base met with varied success, and, although a technique might be developed that would be suitable for routine application in a particular laboratory, the flask-combustion method cannot be recommended as a standard procedure for this type of formulation.

(*vii*) Although the possibility of explosion in carrying out a combustion by the flask-combustion method on a known type of compound is remote, it has been thought wisest to recommend the use of a safety screen and protective gloves or other suitable safety devices.

RESULTS

The results of a final collaborative test, in which the method recommended in the Appendix was used, and which involved some members of the Sub-Committee and other workers in this field who were not familiar with the technique, are recorded in Table I.

TABLE I

RESULTS OF FINAL COLLABORATIVE TEST

Laboratory	Type of finish	Chlorobenzoic acid (Reference Standard) (chlorine = 22·65%*)		P.C.P. miscible oil (chlorine = 9·84%†)		Dieldrin 50% water-dispersible powder (chlorine = 28·0%†)	
		Weight of sample, mg	Chlorine found, %	Weight of sample, mg	Chlorine found, %	Weight of sample, mg	Chlorine found, %
A	Potentiometric	24·0	22·3‡	36·9	9·13	17·7	28·9
		26·8	21·5‡	40·7	9·41	25·5	28·4
B	Potentiometric	26·5	22·6	45·3	9·39	29·6	28·9
		30·5	22·7	49·8	9·40	32·7	28·8
C	Potentiometric	27·9	22·6	45·6	9·49	25·7	28·3
		31·4	22·5	50·4	9·49	26·2	28·1
D	Oxycyanide	30·0	22·5	60·0	9·45	31·2	27·8
		30·7	22·5	61·6	9·32	31·5	28·4
E	Oxycyanide	35·0	22·4	99·4	9·4§	51·8	28·3
		40·9	22·8	94·6	9·6§	45·3	28·3
F	Oxycyanide	28·6	23·1	25·9	11·2	22·0	30·4
		36·5	22·9	40·2	10·8	24·6	29·6

* Theoretical figure, assuming 100 per cent. purity.
† Expected figure, based on formula of preparation.
‡ Own M.A.R. grade material used.
§ A little soot formed on combustion of both oil samples.

An analysis of variance of the results of this collaborative test is shown in Table II.

TABLE II

ANALYSIS OF VARIANCE OF RESULTS FROM FINAL COLLABORATIVE TEST

Source of variation	Including results of Laboratory F		Excluding results of Laboratory F	
	Mean square	Degrees of freedom	Mean square	Degrees of freedom
(1) Between samples	1128·76	2	944·67	2
(2) Between laboratories	8·26	5	0·12	4
(3) Samples × laboratories interaction	0·23	10	0·15	8
(4) Residual	0·07	18	0·05	15
Ratio (2)/(3)	35·9	Highly significant	1	Not significant

The analysis indicates a significant difference in the results obtained between the laboratories, but this difference is removed if the results from Laboratory F are excluded. The mean squares for (samples × laboratories) interaction of 0·23 and 0·15 show that the standard deviation of a single observation, irrespective of the laboratory in which the determination was made or of the chlorine level, is of the order of 0·5 to 0·4.

Appendix

RECOMMENDED METHOD FOR THE SEMI-MICRO-DETERMINATION OF CHLORINE IN ORGANIC SUBSTANCES

PRINCIPLE OF METHOD—

The method is based on the decomposition of the material by combustion in oxygen, with subsequent absorption of the combustion products in neutral peroxide solution and then determination of the hydrochloric acid formed by (*i*) potentiometric titration with silver nitrate solution, (*ii*) the use of mercuric oxycyanide with a comparative end-point procedure or (*iii*) in certain instances, direct alkalimetry.

APPLICABILITY—

The method is applicable to the determination of chlorine in organic insecticides, fungicides, herbicides, etc., in the form of technical chemicals and formulations. The method is not suitable, however, for formulated products containing low levels of chlorine (about 0·5 per cent.) in bases consisting largely of inorganic filler.

RANGE—

For chlorine contents higher than about 5 per cent. (see Note 1, p. 420).

SPECIAL APPARATUS—

Combustion apparatus—This consists of a round flat-bottomed 1-litre flask with a B24 ground-glass neck into which fits a glass stopper of the design shown in Fig. 1 (see Note 2). To the glass extension rod, 3 mm in diameter, is sealed a 30-mm length of platinum wire, 1 mm in diameter, terminating in a 15-mm × 20-mm piece of 36-mesh (or coarser) platinum gauze formed into a cylindrical sheath. The lower end of the sheath should be located centrally about 4 to 5 cm from the base of the flask.

For potentiometric titration—If the potentiometric finish is used, a cell system is required consisting of a glass reference electrode and a silver indicator electrode coupled to an electronic voltmeter or high-impedance pH/mV meter, together with a means for mechanically stirring the titration solution.

REAGENTS—

Absorbent solution—Mix 20 ml of distilled water and 1 ml of 100-volume hydrogen peroxide solution (27 per cent. w/w of H_2O_2) (microanalytical-reagent grade) directly in the combustion flask, and exactly neutralise with 0·02 N sodium hydroxide, with screened methyl red solution as indicator.

Screened methyl red indicator solution—Dissolve 0·125 g of methyl red (free acid; indicator grade) in 50 ml of 90 per cent. ethanol. Dissolve 0·083 g of methylene blue (redox indicator grade) in 50 ml of 90 per cent. ethanol. Store the solutions separately, and mix equal volumes for use as required; the mixed solution keeps for 1 week.

Oxygen.

The additional reagents described below are required if the potentiometric finish is used:

Sulphuric acid, 5 N—Add slowly and with stirring 1 volume of sulphuric acid, sp.gr. 1·84, to 6 volumes of distilled water.

Silver nitrate, 0·02 N—Accurately prepared or standardised.

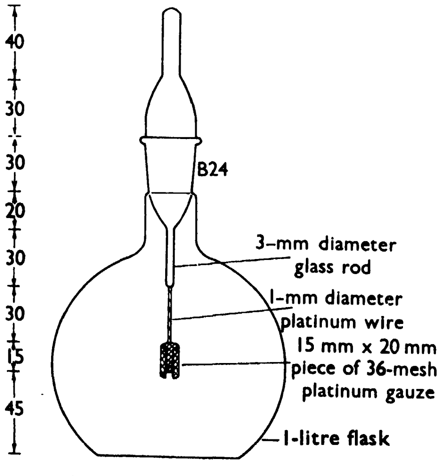
40
30
30
20
30
30
15
45
B24
3-mm diameter glass rod
1-mm diameter platinum wire
15 mm x 20 mm piece of 36-mesh platinum gauze
1-litre flask

Fig. 1. Apparatus for flask-combustion method (all dimensions in mm)

The additional reagents described below are required if the oxycyanide finish is used:

Sodium hydroxide, 0·02 N.

Mercuric oxycyanide solution, saturated—Shake until dissolved 20 g of mercuric oxycyanide with 1 litre of recently boiled and cooled distilled water. Do not heat. Filter the solution, and store in an amber-coloured glass bottle.

Sulphuric acid, 0·02 N.

Standard chloride solution, 0·02 N—Prepare from analytical-reagent grade sodium chloride previously dried at 250° to 350° C for 1 hour.

PROCEDURE

PREPARATION OF SAMPLE FOR COMBUSTION—

Prepare a representative sample of the product for analysis. The weight of sample to be taken for each analysis is indicated below:

Type of sample	Approximate chlorine content, %	Amount of sample needed, mg
Technical chemicals	50	15 to 25
Concentrated powders	25	25 to 35
Emulsifiable concentrates	10	35 to 50
Diluted powders	5	50 to 100

Solid samples—With use of a long-handled weighing tube, weigh the sample on to a piece of Whatman No. 44 filter-paper; use a 3-cm square for samples weighing less than 50 mg and a 4-cm square for samples weighing 50 to 150 mg. Wrap the sample in the paper, and fix the package securely in the platinum-gauze sheath (see Note 3). Handle the paper as little as possible; preferably use rubber finger stalls.

Liquid samples—With use of a capillary dropper, weigh the sample into a hard capsule of gelatin* or, preferably, methylcellulose,† supported on the balance pan in a small glass or metal thimble. Any loss of unburned liquid on combustion of the sample can be prevented by packing the lower half of the capsule with Whatman ashless filter-paper floc before the sample is introduced; about 15 mg of floc is adequate for the absorption of 50 mg of liquid sample. Close the capsule, and fix securely in the platinum-gauze sheath.

COMBUSTION OF SAMPLE—

Insert a filter-paper fuse, 3 mm × 30 mm, into the gauze sheath, set light to it, and quickly insert the stopper into the combustion flask, which contains the absorbent solution and which has previously been flushed out completely with oxygen from a cylinder. Carefully invert the flask so that the absorbent solution forms a seal round the stopper, which should be held firmly in place while the combustion proceeds (see Note 4); protect the exposed hand in a stout canvas glove and interpose a safety screen between the flask and the face.

When combustion is complete, shake the flask intermittently for 10 to 15 minutes to ensure complete absorption of the combustion products. Rinse the stopper, wire and gauze with a little distilled water, collecting the rinsings in the combustion flask, boil the mixed solution and rinsings gently for 1 to 2 minutes to destroy most of the peroxide and expel carbon dioxide, and cool to room temperature.

DETERMINATION OF CHLORIDE—

Determine the chloride in the solution either by potentiometric titration with standard silver nitrate solution or by the mercuric oxycyanide comparative procedure.

Potentiometric Titration

Transfer the boiled and cooled solution from the combustion of the sample quantitatively to a lipless tall-form 100-ml beaker (see Note 5), dilute the solution to about 40 ml with distilled water, and add 20 ml of 5 N sulphuric acid. Stir the solution mechanically, and titrate potentiometrically with 0·02 N silver nitrate added from a 10-ml microburette; use the silver - glass electrode pair. Plot the curve of e.m.f. against volume of silver nitrate solution added, determine the end-point, and calculate the chlorine content of the sample (see Note 6).

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* Suitable capsules (size No. 4) may be obtained from Messrs. Parke, Davis & Co.

† Suitable capsules (size No. 4) may be obtained from A. Gallenkamp & Co. Ltd.

1 ml of 0·02 N silver nitrate ≡ 0·709 mg of chlorine.

Mercuric Oxycyanide Comparative Procedure

To the boiled and cooled solution from the combustion of the sample (see Note 5) add screened methyl red indicator solution, and neutralise with 0·02 N sodium hydroxide, matching the colour with that of a control solution consisting of an equal volume of neutralised freshly boiled and cooled distilled water containing the same amount of screened indicator solution in a similar flask (see Note 7). Add 20 ml, or more if necessary (see Note 8), of saturated mercuric oxycyanide solution to the test solution, and titrate the liberated alkali with 0·02 N sulphuric acid until the colour again matches the neutral shade of the indicator in the control solution.

To the control solution add the same volumes of saturated mercuric oxycyanide solution and 0·02 N sulphuric acid as were added to the test solution, and titrate the mixture with 0·02 N standard chloride solution until the colour matches that of the test solution; at the end-point, the volumes and the temperatures of both test and control solutions must be the same. Calculate the chlorine content of the sample from the volume of standard chloride solution added (see Note 9).

1 ml of 0·02 N standard chloride solution = 0·709 mg of chlorine.

Alternative Direct Alkalimetric Finish

If a gelatin capsule has not been used and the sample contains no other interfering elements or components, *e.g.*, nitrogen, sulphur or alkalis, the hydrochloric acid in the boiled and cooled solution from the combustion of the sample may be titrated directly with 0·02 N sodium hydroxide and screened methyl red indicator solution.

BLANK TESTS—

Carry out blank determinations on appropriate amounts of filter-paper floc and on the capsules. Significant blank values are to be expected on some batches of gelatin capsules.

NOTES

1. For some routine purposes, when high precision is not essential, the method may be applied to materials containing less than 5 per cent. of chlorine. When greater precision is required with such materials, the Stepanow procedure (as given in "Specifications for Pesticides," World Health Organisation, 1956) is suitable.

2. For the combustion of solid samples, a 500-ml conical iodine flask is satisfactory. Care must be taken to avoid soot formation.

3. Normally, the sample can be wrapped in the middle of the paper, which is folded into three and then doubled over. For the heavier samples, when much inorganic matter is present, better combustion may be achieved by spreading the sample carefully over the paper before it is folded, so that interleaving of the sample and paper takes place. For samples that burn only with difficulty, the use of filter-paper pre-treated with a strong solution of potassium nitrate and then dried may be of advantage.

4. The flame of the burning sample should not impinge on the glass, as unburned carbon may be produced and in such a determination low results may occur. Should soot formation occur, the distance between the gauze and the bottom of the flask may be increased. Soot formation may also occur if the flask has not been adequately flushed out with oxygen.

5. If unburned carbon or insoluble alkaline residue (from inorganic filler) is present, this should be filtered off, preferably by the use of a sintered-glass funnel.

6. Initially, a full plot of e.m.f. against silver nitrate titre will be necessary to establish the e.m.f. of the titration end-point. Thereafter, however, it is possible to titrate directly to the pre-determined e.m.f. and read off the end-point titre from the burette. The end-point e.m.f. should be re-determined whenever the electrodes are cleaned.

7. If the material contains an alkaline filler, the solution after filtration (see Note 5 above) will be alkaline and must be neutralised with acid.

8. An excess of oxycyanide solution must be used to allow the reaction to go to completion. For 0 to 12 mg of chloride, use 20 ml of saturated mercuric oxycyanide solution and for 12 to 20 mg use 30 ml.

9. Experience may show that on this scale of working the chlorine content of the sample, calculated from the direct sulphuric acid titration of the alkali produced by addition of oxycyanide to the test solution, is identical with that obtained by the comparative procedure given above. Confirmation of this fact should allow the comparative titration procedure to be replaced by a simpler direct oxycyanide titration method.

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Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factors for Beef

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams (deputy Mr. H. C. Hornsey), with Dr. C. H. Tinker as Secretary.

In its report on "Nitrogen Factors for Pork,"[1] the Sub-Committee expressed its view that Stubbs and More's method[2] for determining the raw fresh meat content of manufactured products was the best one currently available. There was, however, evidence that the nitrogen factors hitherto used were not altogether valid at present.

In 1952 the Analytical Methods Committee[3] recommended a factor of 3·4 for beef. The Sub-Committee came to the conclusion that too few reliable results had been published[2,4,5] to permit a critical review of the factor. The Sub-Committee was fortunate in again securing the collaboration of various meat-product manufacturers and meat-research organisations (see p. 423) all over the world in determining the nitrogen content of fresh beef meat in accordance with its requirements.

All the results collected by the Sub-Committee are shown in Fig. 1. Figures quoted in the literature relating to individual muscles are not included, as they were not considered

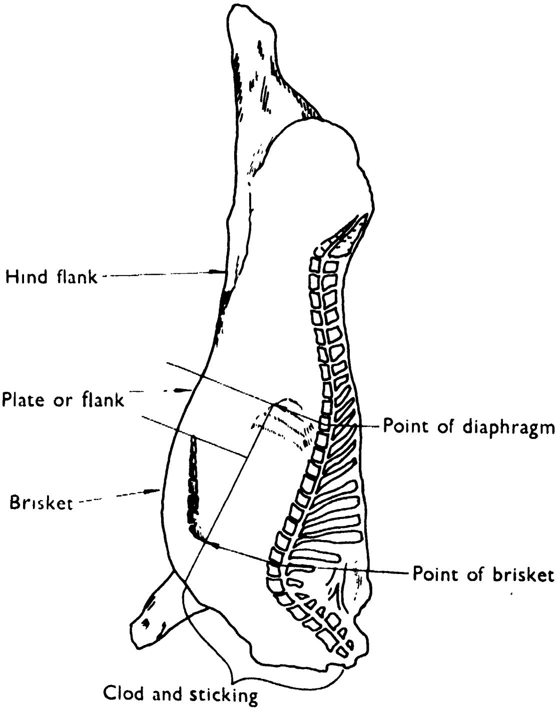

Fig. 2. Diagram showing factory cuts of beef used in collaborative tests

relevant to the investigation. To reduce sampling errors as much as possible the Sub-Committee asked for analyses to be carried out by one of two recommended methods. In the first, certain cuts of beef (as marked in Fig. 2, which was sent out to all collaborating laboratories) were to be comminuted, mixed and sampled for analysis. The selected cuts were those known to the trade as factory cuts, *i.e.*, flank, shin, plate, brisket and clod with sticking. In the second, all usable meat from the whole carcase was to be comminuted, mixed and sampled for analysis. As the latter method was costly and laborious, only 16 carcases were analysed in this way.

In reviewing all the available results, the Sub-Committee noticed an interesting distribution of figures according to the country of origin (see Table I). Some loss of moisture in carcases transported over long distances was indicated by the slightly higher average nitrogen content of meat from the Argentine and Australia when analysed in this country.

TABLE I

SUMMARY OF NITROGEN (FAT-FREE) VALUES

	At country ot origin, %	After import to U.K., %
Home killed	3·6	—
Argentine	3·4	3·5
Australian	3·7	3·8
Danish	3·45	—
Dutch	3·5	—
German	3·6	—
Hungarian	3·6	—

As found in the previous work on the nitrogen factor for pork, a wide range of values was also obtained for beef, namely, 2·96 to 4·53. The arithmetical mean for all the figures (716 samples) was 3·57, whereas that for chilled and frozen samples only was 3·58 (32 samples). For 16 whole carcases the average value was 3·59, for the factory cuts 3·56 (223 samples) and for the miscellaneous samples, which also represented types of meat used for manufacturing purposes, 3·53 (260 samples).

RECOMMENDATION

After due consideration of the types of beef used in the manufacture of comminuted products, the Sub-Committee recommends an average nitrogen factor of 3·55 as the best compromise for general use.

ACKNOWLEDGMENT

The Sub-Committee thanks those listed below for their help and communications—

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N. V. H. Hartog's Fabrieken Oss, Holland.
Oxo Ltd.
J. Sainsbury Ltd.
T. Wall & Sons Ltd. (Meat & Handy Foods) Ltd.

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Silver- and Halide-ion Responsive Electrodes

Part I. Preparation, Treatment, Durability and Zero-current Response in Aqueous Media

By E. BISHOP and R. G. DHANESHWAR*

(*Washington Singer Laboratories, The University, Exeter*)

In the preparation and treatment of silver metal, silver halide, gold and silver amalgam and cation-responsive glass electrodes, the current density, electrolyte composition and duration of deposition of halide films, the conditioning of electrodes before use, the durability of the electrodes and methods of cleaning and storage have been studied, and recommendations are made. The response of the electrodes to silver, chloride, bromide and iodide ions in aqueous solutions in the concentration range 10^{-1} to 10^{-9} M has been determined under dynamic conditions in the absence of supporting electrolytes, and values of formal potentials, slope factors, response ranges and failure points have been derived. All the electrodes respond first order to silver ion, with efficiencies of 65 to 75 per cent. Silver, clean or fouled, amalgam and halide-coated electrodes, provided the coating is of a halide of atomic number equal to or greater than that of the halide ion in solution, behave as halide electrodes with greater than theoretical slopes. Silver amalgam behaves as an active silver metal electrode, gold amalgam as a mercury electrode.

In studies of the differential electrolytic potentiometry (DEP)[1] of argentimetric reactions, phenomena have been encountered[2,3,4] that are not explicable by classical theories of electrode behaviour. From the behaviour patterns of DEP, electrode behaviour can be deduced with some confidence, but confirmation is lacking. Despite a tacit but vague agreement among electrochemists that silver electrodes respond to halide ions, halide electrodes respond to silver ion, slopes may deviate from theoretical and electrodes become non-Nernstian at high dilution, recourse to the literature reveals little supporting evidence. The preparation, reproducibility, surface characteristics and standard potential are abundantly covered for silver chloride electrodes, and adequately for silver bromide[5] at 0·1 M and above. Apart from a study of the hydrosulphide and silver ion responses of silver metal[6,7] attention has been confined[8,9] to silver concentrations $> 10^{-3}$ M. Dual response of electrodes has had scant notice.[6,10,11] The influence of bromide ion on chloride electrodes has been noted[12] and general directions for the analysis of halide mixtures have been given,[13] but no information is available on halide interaction. Electrolytic pre-treatment of electrodes has been mentioned,[14] but neither the effect of continuous passage of current nor behaviour in mixed solvent - water media at lower concentrations has been treated. Electron-transfer systems affect potentiometric response in argentimetry,[15] but no quantitative study has been reported. The non-theoretical behaviour of many electrodes is attested by the limited usage of absolute potentiometry. Relative potentiometry, such as null-point potentiometry[16] or conventional pH measurement, also suffers limitations. In summary, most publications deal with the performance of silver and halide electrodes at high constant concentration in respect of their function as reference electrodes, and not with their indicator response characteristics.

To set the subject on a quantitative basis and to confirm and extend the interpretation of argentimetric DEP, a complete investigation by the method of response curves has been undertaken. This paper deals with the preparation, treatment and durability of a wide range of electrodes and their responses in aqueous media in the absence of supporting electrolytes. Part II deals with behaviour in solvent - water media and with the effect of polarising currents; the final part treats ionic interactions and supporting electrolytes.

EXPERIMENTAL

ELECTRODES—

Wire electrodes were made from 22 s.w.g. mint silver or fine gold (Johnson, Matthey & Co. Ltd.) sealed into soft glass sheaths as previously described[17] and cut to size. Micro electrodes[2] were made from the same wire, cut at the seal and ground square to expose the cross-section of the wire (0·003973 sq. cm). Square sheet silver electrodes of side 1, 0·316 and 0·2 cm cut to a precision of ±0·1 per cent. were mounted by welding to 22 s.w.g. wire and sealing into the shrunk end of a glass sheath by means of Araldite so as to avoid heat distortion, and the backs and edges coated with Araldite so as to expose areas of 1, 0·1 and 0·04 sq. cm. Wire-form silver metal electrodes were trimmed to 1·5 cm, coated wires were trimmed to 2 cm before coating.

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Silver halide electrodes were prepared by making cleaned silver wire electrodes anodic against a silver cathode in the appropriate electrolyte (100 ml of 0·75 M hydrochloric acid, 0·5 M hydrobromic acid or 0·05 M potassium iodide *plus* one drop of 0·01 M sulphuric acid) at the optimum current density for the required time (20 mA per sq. cm for 45 minutes for chloride, 0·2 mA per sq. cm for 4 hours for bromide or 2 mA per sq. cm for 15 minutes for iodide), with subsequent thorough washing and immersion in 0·01 M halide until the potential reached equilibrium.

Silver and gold amalgam electrodes were prepared by immersing the mounted and carefully cleaned wire in Specpure mercury for a few seconds. Cation-responsive glass (BH68) electrodes, kindly supplied by Electronic Instruments Ltd., Richmond, Surrey, as GNA23 electrodes, were transported in 0·1 M sodium chloride, rinsed with water and aged in 0·1 M silver nitrate for 8 days before use. They should not be allowed to dry out. The reference electrode was a high capacity mercurous sulphate - saturated potassium sulphate cell[15] connected to the test solution via a saturated potassium sulphate salt bridge terminated by a low leakage rate ceramic plug.

REAGENTS—

All reagents were of AnalaR grade and were analysed as previously described.[2,17] Quartz-distilled water[18] of halide content $< 10^{-11}$ M was used throughout. Transistor-grade nitric acid was used for cleaning the electrodes. Standard 0·1 M silver and halide solutions were prepared by direct weighing, and used as stock. More dilute solutions were freshly prepared before use by successive ten-fold dilution of the stock solutions in calibrated glassware.

PROCEDURE—

After suitable cleaning or pre-treatment, sets of the various electrodes were immersed in the appropriate vigorously stirred solution at 20° C and their potentials with respect to the reference electrode observed serially at intervals on a Doran M4989 potentiometric pH meter until the rate of drift became imperceptible (below 0·1 mV per minute). After the electrodes had been thoroughly washed, the next more dilute solution was substituted, and the process repeated until the potential showed no further change with dilution. The process was then repeated in reverse order.

PREPARATION, TREATMENT AND DURABILITY OF THE ELECTRODES

INFLUENCE OF CURRENT DENSITY IN THE PREPARATION OF HALIDE ELECTRODES—

Current density affects the nature and electrical properties of the deposit.[19] The method for chloride, based on that of Bates,[20] proved quick and reliable, and gives reproducible electrodes, stable for considerable periods. The initial current density of 20 mA per sq. cm falls to 15 mA per sq. cm. Above 18 mA per sq. cm a white deposit of poor adhesion is formed[19]; at lower current density the mechanism of deposition changes, resulting in a porous adherent deposit. Jaenicke, Tischer and Gerischer,[21] studying the cathodic reduction of chloride films, found that the ohmic resistance of the film rapidly drops as silver metal forms along the inner crystal grain boundaries. The chloride film on a micro cathode spalls under conditions of excessive differentiating current density, when a loose or easily attackable boundary film of silver is produced, or in too strongly acid conditions when the fresh metal is rapidly attacked.[2]

Deposition of bromide by this method failed because the current rapidly decayed virtually to zero. Apparently,[22] at 20 mA per sq. cm a non-porous high-resistance film forms and stops the flow of current. This high current density proved usable if the hydrobromic acid concentration was increased to 10 M, but a non-uniform non-adherent deposit resulted. Prolonged deposition at low current gave excellent electrodes. Rather higher currents and lighter deposits gave the most satisfactory iodide films. Thiocyanate is best deposited at 18 mA per sq. cm for 2 hours, with continuous passage of nitrogen, from 100 ml of 0·1 M potassium thiocyanate containing 1 ml of 72 per cent. perchloric acid and previously de-oxygenated with nitrogen. Reduction of the current density to 12 mA per sq. cm gave a bulky dark grey easily removable deposit instead of the uniform adherent dull white film.

AGEING OF ELECTRODES BEFORE USE—

Little has been published on electrode ageing other than on silver chloride.[23,24] Ageing in aerated solutions has been attributed to—

$$2Ag + 2Cl^- + 2H^+ + \tfrac{1}{2}O_2 = 2AgCl + H_2O$$

causing a slight decrease in chloride concentration within the pores of the deposit giving a potential slightly positive to that of an air-free electrode. This concentration polarisation may take up to 2 days to vanish. Although the work described here confirms that fresh electrodes are positive to aged electrodes, the role of oxygen is uncertain. A 48-hour pre-ageing treatment of chloride electrodes in 0·01 M potassium chloride has been made standard practice. Thiocyanate electrodes require ageing for 24 hours in 0·1 M thiocyanate solution. In contrast, bromide electrodes reached equilibrium within 2 hours, and iodide electrodes stabilised within a few minutes. Stabilisation of coated electrodes to silver ion is similar. Except for thiocyanate, the ageing period bears some relation to the durability of the electrodes.

Silver metal electrodes give an immediate proper response to both silver and halide ion, and the potential in halide media agrees closely with that of an aged halide-coated electrode. Both silver and gold amalgams rapidly reach equilibrium in chloride and bromide media $> 10^{-5}$ M and in silver solutions $> 10^{-2}$ M. However, a considerable period of ageing is required before their silver ion response extends to 10^{-6} M. Silver-responsive glass requires conditioning in 0·1 M silver nitrate for at least 8 days.

DURABILITY OF ELECTRODES—

Silver metal is readily reactivated (see below), but other electrodes become "poisoned," presumably by clogging of the pores of the deposit. Among factors contributing to this de-activation are light, oxygen, impurities and the nature and composition of the solvent medium. Thermal cycling and degree of use also modify the structure of the deposit. Response was checked daily before use and the period of time noted when response became sluggish or the potential or slope deviated from normal. With proper care and storage, chloride electrodes remained active for 21 days, bromide electrodes for 5 to 6 days, but iodide electrodes became inactive after 36 hours and thiocyanate after 48 hours. Amalgam electrodes maintain activity for 6 months or more.

CLEANING AND STORAGE OF ELECTRODES—

In use, electrode performance, particularly in dilute solution, gradually becomes impaired by sluggish response and shortening of response range. The effect of different cleaning agents on the potential of a silver electrode in 10^{-3} M silver solution is shown in Table I. The electrodes were fouled by immersion for 2 hours in a halide solution before, and washed thoroughly after, they had been cleaned.

TABLE I

EFFECT OF CLEANING AGENTS ON THE POTENTIAL OF A SILVER ELECTRODE IN 10^{-3} M SILVER SOLUTION

Agent	Deviation from expected potential, mV
Potassium cyanide, 1·0 M	−33
Sodium hydroxide, 10 M	−123 (immediately) −63 (after 30 minutes)
Nitric acid, concentrated	+30 (immediately) 0 (after 2 hours)
Nitric acid (1 + 1)	0
Abrasion with No. 0 emery paper ..	−5

Cyanide removes grease, but chemical or mechanical exposure of a fresh metal surface is best. Immersion in aqua regia serves for treating gold before amalgamation; chromic - sulphuric acid is also effective, but may lead to oxidation or chemical contamination resistant to washing. Abrasion with emery paper is effective, but liable to deform the metal surface, induce strains and mechanically contaminate the electrode; it is not recommended. Immersion in (1 + 1) nitric acid and then washing for 5 minutes with distilled water has been adopted as the standard treatment. Increased nitric acid concentration leads to high potentials that require 2 hours to return to normal; excess of silver ion accumulates in the exchange layer and diffuses only slowly into the solution.[25]

Unless stripped and re-coated, halide-coated and amalgam electrodes cannot be chemically cleaned, and can be washed only with water, so it is essential that they be properly stored when not employed and not be allowed to dry out. Electrodes are best stored in the medium in which they are to be used, but water serves well. Amalgam electrodes dried in air for an hour or two suffer a change in slope and response range, and display potentials more negative throughout. After exposure to organic solvents, halide-coated electrodes especially require very prolonged washing with water before the potentials return to normal.

ELECTRODE RESPONSE

For a reaction—

$$B^+ + A^- \rightleftharpoons BA$$

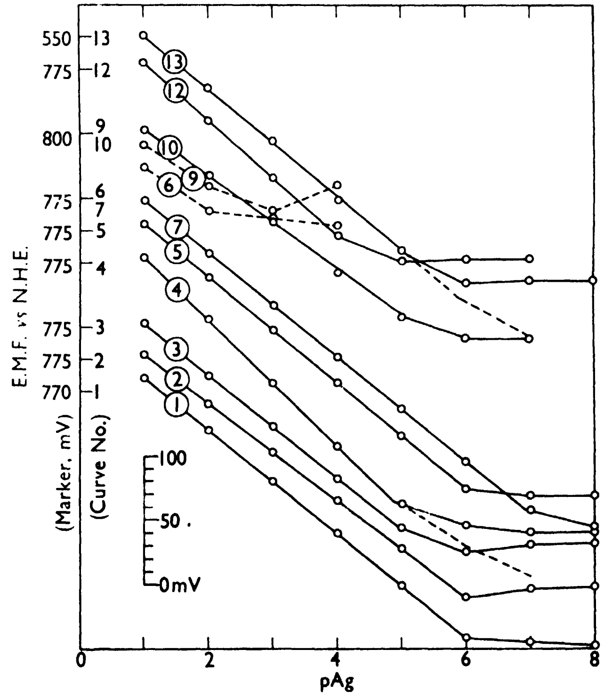
an electrode responding to the ion B^+ gives a potential—

$$E = \pi_0 + S\log[B^+] = \pi_0 - SpB$$

and an electrode responding to the ion A^- gives a potential—

$$E = \pi_0 + S\log\frac{1}{[A^-]} = \pi_0 + SpA$$

where π_0 is the formal potential at unit ionic concentration and S is the slope factor. A plot of potential versus ion exponent is a straight line of slope S, making an intercept π_0 at pB



KEY TO CURVES—

1. Silver cleaned in nitric acid (1 + 1)
2. Silver fouled with chloride
3. Chloride-coated silver
4. Bromide-coated silver
5. Iodide-coated silver
6. Freshly amalgamated silver
7. Aged silver amalgam
8. Aged silver amalgam allowed to dry in air
9. Freshly amalgamated gold
10. Aged gold amalgam
11. Aged gold amalgam allowed to dry in air
12. Micro silver metal,[3] 0·004 sq. cm
13. BH68 glass

Fig. 1. Response to silver ion

or pA = 0. As pB or pA increases, a point is reached where the slope changes, usually the graph begins to curve; this point defines the lower concentration limit of linear response. Thereafter, the response continues on further dilution with a much reduced or continually changing slope for a further one or two pB or pA units until the failure point, beyond which the potential becomes constant or reverses its direction of change. Occasionally, an intermediate slope is interposed between the initial and final slopes. The theoretical equivalents of π_0 and S are the normal potential E_0 and the Nernst factor 2·303 RT/nF. Deviation of experimental from theoretical values will be discussed later; the experimental values presented here are not necessarily true thermodyanamic equilibrium values, but rather dynamic values designed to relate to the requirements of titrimetric practice. Particularly at extremely low concentrations, potentials continue to drift away from theoretical for 300 to 400 hours,. approaching ultimate equilibrium asymptotically, the last few millivolts occupying 99 per cent. of the time. Titrimetrically, a drift rate less than 1 mV per minute is usually acceptable; 0·1 mV per minute is entirely adequate. The latter criterion has been adopted here; frequent checks showed the drift to be less than 1 mV per hour. The term "dynamic" is chosen in distinction from "thermodynamic" and in contrast to "kinetic" or instantaneous measurements (*e.g.*, the dotted extension of curves (4) and (13) in Fig. 1), which have special virtues to be described elsewhere.

INDIVIDUAL RESPONSE CURVES—

Little stress is laid in the literature on the response of silver electrodes to halide ions and even less on the response of halide-coated electrodes to silver ion.[26] Amalgam electrodes appear to have escaped notice.[6,10,11] Electrodes tend to respond to silver or halide ion depending on which is in excess, and change in nature during an argentimetric titration. This accords with the shape of recorded titration curves,[17] which indicate a change of slope during titration.

Response of various electrodes to the four ions, silver, chloride, bromide and iodide, in aqueous solution free from supporting electrolyte are depicted in Figs. 1 to 4. Many unequivocal sharp discontinuities in slope occur, but curvature is not excluded by discontinuous presentation. Critical values of π_0, S, limits of linear response and failure points are

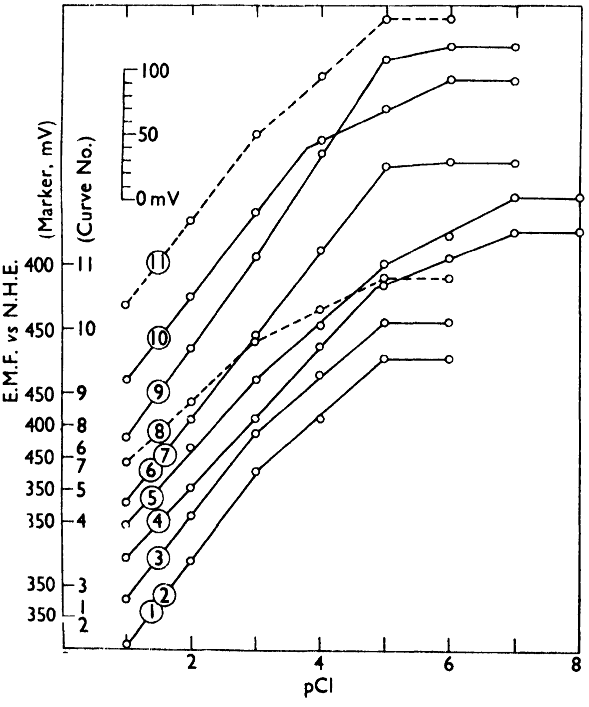


Fig. 2. Response to chloride ion (for key to curves, see Fig. 1)

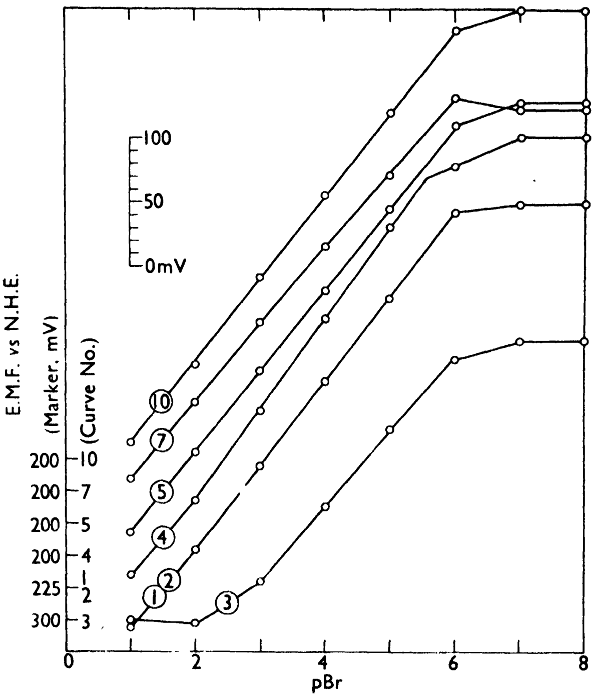


Fig. 3. Response to bromide ion (for key to curves, see Fig. 1)

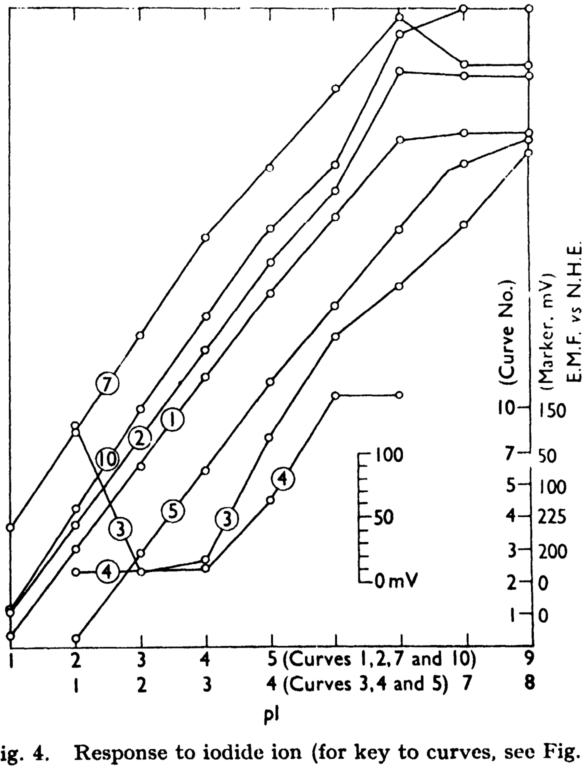


Fig. 4. Response to iodide ion (for key to curves, see Fig. 1)

TABLE II

RESPONSE OF ELECTRODES TO SILVER AND HALIDE IONS IN AQUEOUS SOLUTION AT 20° C

Electrodes	(1) Ag (clean)	(2) Ag (dirty)	(3) AgCl	(4) AgBr	(5) AgI	(6) Silver amalgam (fresh)	(7) Silver amalgam (aged)	(8) Silver amalgam (dried)	(9) Gold amalgam (fresh)	(10) Gold amalgam (aged)	(11) Gold amalgam (dried)	(12) Micro Ag	(13) BH68 glass
Response to Ag^+—													
π_0 (extrapolated), V	0·820	0·817	0·818	0·827	0·821	0·832	0·814	—	0·828	0·840	—	0·825	0·594
Initial slope, mV/pAg	40·5	38	40·0	48·0	41·6	—	40·8	—	—	36·0	—	45·7	41·8
Limit, pAg	6	6	4·9	4·9	6	2	6·9	—	2	5·1	—	4	5·1
Final slope, mV/pAg	—	—	18	17	—	—	12	—	—	17	—	20	22
Failure point, pAg	6	6	6	6	6	2	8	—	3	6	—	5	6
Response to Cl^-—													
π_0 (extrapolated), V	0·260	0·262	0·2755	0·2677	0·266	0·350	0·350	0·323	0·345	0·345	0·301		
Initial slope, mV/pCl	68	68	64·5	54·3	56·5	65	65	48·5	70	65	66		
Limit, pCl	3	3	3	4·85	3	5	5	3	3	3·8	3		
Intermediate slope, mV/pCl ..	—	—	—	—	45	—	—	—	78·5	—	—		
Limit, pCl	—	—	—	—	5	—	—	—	5	—	—		
Final slope, mV/pCl	44	44	42·5	20·5	26	—	—	23·5	10	23·5	45		
Failure point, pCl	5	5	5	7	7	5	5	5	6	6	5		
Response to Br^-—													
π_0 (extrapolated), V	0·136	0·136	(0·155)	0·126	0·129	—	0·149	—	—	0·149	—		
Initial slope, mV/pBr	59	59	—	59	63·4	—	60·2	—	—	64·2	—		
Limit, pBr	2	2	—	2	6	—	4	—	—	6	—		
Intermediate slope, mV/pBr ..	66·7	66·7	55	71	—	—	—	—	—	—	—		
Limit, pBr	6	6	3–5·9	5·6	—	—	—	—	—	—	—		
Final slope, mV/pBr	5	5	14	22	18	—	58·2	—	—	16	—		
Failure point, pBr	7	7	7	8	8	—	6	—	—	7	—		
Response to I^-—													
π_0 (extrapolated), V	−0·082	−0·088			−0·084	—	−0·077	—	—	−0·083	—		
Initial slope, mV/pI	67	67·3	erratic		66·3	—	72	—	—	77·8	—		
Limit, pI	4·7	5			4	—	4	—	—	3	—		
Intermediate slope, mV/pI ..	—	—	erratic		59·5	—	—	—	—	70	—		
Limit, pI	—	—			6·8	—	—	—	—	4·9	—		
Final slope, mV/pI	60	erratic	erratic		20	—	57	—	—	erratic	—		
Failure point, pI	7	7	8	5	8	—	7	—	—	8	—		

presented in Table II. Potentials have been corrected to the hydrogen scale. The kinetic, or instantaneous, value of the potentials in any given medium approaches more nearly the theoretical or straight line value at high dilution (compare the dotted extensions to curves (4) and (13) in Fig. 1), and the drift is away from theoretical, is unidirectional and does not recover and reverse after prolonged equilibration. The deviation is a reduction in slope, ultimately to zero, and cannot be due to loss of ions from solution by adsorption,[2] because this would cause a drift in the opposite direction, *i.e.*, towards a lower than apparent concentration.

CONCLUSIONS

1. All except the freshly amalgamated electrodes respond first order to silver ion similarly to silver metal. The different π_0 value for BH68 glass is admissible, because this membrane electrode responds to univalent cations and is not specific to silver ion Aged amalgamated silver electrodes give the best performance.

2. Silver ion response slopes are all considerably less than theoretical, showing efficiencies of 65 to 75 per cent., and do not reveal any intermediate changes.

3. Silver metal electrode response to silver ion is independent of electrode area down to 0·04 sq. cm; reduction of the area to 0·004 sq. cm impairs the response range but improves the slope.

4. All electrodes except glass respond similarly to halide ions, provided the halide coating has an atomic number equal to or greater than that of the halide ion in solution. Amalgam electrodes are exceptional only in respect of the π_0 values in chloride media. Bromide-coated electrodes give the best response to chloride ion, and iodide-coated electrodes to bromide ion.

5. Halide ion initial response slopes are generally greater than theoretical, and intermediate changes are frequent.

6. Fouled silver electrodes show a slightly sluggish and reduced silver ion response, but their halide ion response is practically identical to that of clean silver and silver coated with the relevant halide. Combined with other evidence[1,2,3] this indicates that even a zero-current metal electrode acquires an adherent halide coating in halide medium. Moreover, a silver electrode used to determine, *e.g.*, bromide response, when then used to determine silver ion response gives a curve exactly superimposable on curve (4) of Fig. 1.

7. A halide-coated silver electrode responds to both silver ion and its own halide ion; it responds even better to a halide ion of lower atomic number, and its response to a halide of higher atomic number indicates that a slow exchange of halide occurs between the solid and solution phases resulting in an electrode of mixed and erratic behaviour. This change in surface nature has been seen taking place.

8. To silver ion, fresh amalgam electrodes show a vestigial response that improves on ageing, aged silver amalgam giving the best response of all. This may be ascribed to migration of silver atoms from the base metal to give an activated surface. Freshly amalgamated gold and silver show a response due to kinetic exchange between mercury atoms and silver ions, which lose their charge and migrate into the amalgam faster than they reach the surface from the solution when this is dilute. The aged gold amalgam differs a little from the silver amalgam in π_0 and slope, but why it should behave differently from a freshly amalgamated gold electrode is obscure. Were the electrode aged in a silver ion solution, an exchange between mercury and silver ion would reach equilibrium and a silver ion response would be expected. It appears that growth of the gold atom population in the amalgam surface on ageing slows down migration of silver atoms away from the surface sufficiently to give silver ion response characteristics. The π_0 values indicate that all electrodes respond first order to silver ion, independently of electrode nature.

9. It is to be expected that amalgam electrodes should respond to halide ion and that the response should be better than that of a silver electrode, because of the more favourable formation constants of the mercurous halide. Although the overpotential $\pi_0 - E_0$ of about 80 mV is rather high, chloride response of freshly amalgamated electrodes is ascribed to the reaction $2Hg + 2Cl^- \rightleftharpoons Hg_2Cl_2 + 2e$, because the silver ion response of silver amalgam electrodes shows that silver electrode kinetics is not established until the electrode is aged. Aged silver amalgam shows a failure point of pCl 5 and behaves as a mixed electrode, whereas

aged gold amalgam with a failure point of pCl 6 and a modified slope is a mercury electrode. Aged silver amalgam shows the characteristics of an active silver electrode in bromide and iodide media, and aged gold amalgam acts as a mercury electrode giving good behaviour in bromide media and the expected erratic response in iodide media. This interpretation is supported by the π_0 values and the behaviour of the electrodes in titrations.

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Silver- and Halide-ion Responsive Electrodes

Part II. The Behaviour in Mixed Solvent - Water Media, and the Effect of Restoring Currents on Electrode Response

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A selection of suitable solvents is made on a basis of titrimetric performance and potential shift of chloride ion response. Potential shift increases with solvent concentration, rapidly above 80 per cent., and with decreasing ionic concentration. Fouling, or even poisoning, of electrodes by solvent adsorption is pronounced and difficult to remove. Dynamic zero-current response curves and formal potentials, slope factors and failure points are recorded for clean silver, silver fouled with chloride, silver chloride, silver amalgam and gold amalgam electrodes responding to silver ion in 80 per cent. methanol and acetone, and to chloride ion in 80 per cent. methanol. Acetone gives rise to remarkable responses and severe electrode poisoning. Restoring currents extend the linear silver ion response of macro electrodes to pAg = 11·7, of micro electrodes to pAg = 10·7, and the chloride ion response of macro electrodes to pCl = 8·7. The silver ion restoring current increases with pAg to a sharp maximum and then declines, in the same manner as does the double-layer differential capacitance. The function of the current is therefore to charge the double-layer capacitor. The chloride current demand is many times greater than for silver, and attains a stationary maximum. The effects of counter-, pre-, time-decay and induced polarisation are demonstrated. The results are used to account for phenomena encountered in potentiometric and differential electrolytic potentiometric titrimetry.

In Part I[1] of this series the preparation, conditioning, storage, cleaning and durability of electrodes responding to silver ion and halide ion were described. Zero-current response curves in aqueous media free from supporting electrolyte were presented for clean and dirty silver metal, silver chloride, silver bromide, silver iodide, silver amalgam, gold amalgam and cation-responsive membrane (BH68 glass) electrodes, from which values of dynamic formal potentials and slope factors were derived. In argentimetric titrimetry at titrant concentrations $\leqslant 10^4\, S^{\frac{1}{2}}_{AgX}$, organic solvents or solvent - water mixtures are beneficial, primarily on account of the resultant depression of the solubility of the silver halide. Studies of such systems,[2] and of the differential electrolytic potentiometry (DEP) of argentimetric reactions at high and extreme dilution on both micro[3] and macro[4] scales revealed phenomena inexplicable from theoretical Nernstian electrode behaviour, but understandable from the behaviour pattern of DEP.[5,6] Such deductions required confirmation, and further information on electrode behaviour at low ionic concentration in mixed solvents was desirable. Search of the literature revealed some work on concentrated solutions and on standard potentials,[7,8] but nothing on low concentrations and little on amalgam electrodes.[9] Consequently, an investigation was undertaken of the behaviour of metal, typical coated metal and amalgam electrodes in mixed solvent - water media, free from supporting electrolytes, both at zero current and under the influence of restoring currents.

EXPERIMENTAL

The preparation and treatment of the electrodes and the determination of response curves have been described.[1,2] Polarisation was effected according to the requirements of DEP, *i.e.*, the currents were heavily stabilised by high value series ballast resistors such that the product of source voltage and ballast resistance fell within the range 10^{10} to 10^{14} volt-ohms, by methods previously described.[10,11] All potentials have been converted to the hydrogen scale. Electrodes are numbered to correspond with Part I.[1]

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NATURE AND CONCENTRATION OF THE SOLVENT—

Selection of solvent—Primarily, the solvent must be miscible with water and must exert the greatest effect on the electrode potentials without making them unstable. Exploratory work indicated that a solvent to water ratio of 80 to 20 was advantageous. Chloride ion responses were therefore taken in a mixture of 20 ml of 0·1 M potassium chloride and 80 ml of solvent. The more interesting results are recorded in Table I. Acetone and methanol give the best shifts and dioxan causes instability, in agreement with titrimetric findings.[3,4] All solvents cause negative shifts, an effect in terms of aqueous response equivalent to increasing the chloride concentration by up to three orders of magnitude.

TABLE I

ELECTRODE POTENTIALS VERSUS N.H.E. IN 2×10^{-2} M CHLORIDE IN 80 per cent. v/v SOLVENT - WATER MEDIA

		Electrodes and potentials in millivolts				
Solvent	Dielectric constant ϵ	(1) Ag	(2) Ag dirty	(3) AgCl	(7) Ag/Hg	(10) Au/Hg
Water	80·4	375	375	385	460	455
Methylated spirit ..	—	295	302	310	307	322
Ethylene glycol ..	37	338	340	339	335	361
Ethanol	24·3	264	302	302·5	297	309
Dioxan	2·2	208	251	263·5	253	277
Acetone	20·7	193	228	278	233	240
Methanol	33·6	155	259	322	320	314

Electrode fouling—Clean and halide-fouled (*i.e.*, simply washed with water after exposure to a halide solution) silver metal electrodes differ little in response in water,[1] but differ considerably in solvent media. Solvent-fouling is even more pronounced; a silver metal electrode transferred from the ethanol solution to the methanol solution with an intervening thorough wash with water, showed a potential of 312 mV, and only after it had been cleaned three times in (1 + 1) nitric acid was the stable potential of 155 mV reached. Because halide-coated or amalgam electrodes cannot be chemically cleaned, adsorbed solvent molecules are not easily removed and reproducibility is impaired, in the extreme to the extent of ±15 mV. The silver metal electrode is therefore a more attractive indicator electrode in such media.

The proportion of solvent—The effect can be illustrated in two ways, for changing and for constant formal ionic potential. In Fig. 1, the blocks of curves (A) for silver ion response and (C) for chloride ion response were taken in solutions of x ml of 10^{-1} M ion diluted to 100 ml with methanol, so that $100 - x$ represents the percentage of methanol; curves (1a) and (1c) show the response on dilution with water. With decrease of formal concentration, the silver ion potential instead of falling as in curve (1a) actually rises, and the chloride ion potential instead of rising as in curve (1c) actually falls, *i.e.*, the potentials move in the direction of the apparently increasing concentration, and this contrary movement is more marked for chloride than for silver ion. All curves show a break at 80 per cent. of solvent, and if replotted in terms of deviation from aqueous response show a marked steepening at this point. This indicates the condition of substantial titrimetric benefit combined with economy. The effect, moreover, increases with decreasing ionic concentration as well as with increasing solvent proportion, and it is profitable[3] to increase the proportion of solvent to 95 per cent. in micro titrations at extreme dilution.

The effect at a constant formal concentration of 10^{-3} M is shown in block (B) of Fig. 1 for the silver ion response of silver metal (1b) and silver bromide (4b) electrodes, and increases rapidly in methanol proportions above 80 per cent. For chloride ion response, the normal potential E_0 of a silver chloride electrode[12] is plotted on a solvent concentration (w/w) scale in block (D), along with the dielectric constant, ϵ, of methanol - water mixtures[12] on the same scale.

The behaviour indicates a drastic change in solvation of both active ions and electrode surface; adsorption has already been mentioned. Free energy of solvation has been recognised as a factor in electrode behaviour in mixed solvent media,[13,14] but no distinct relation with dielectric constant can be extracted.

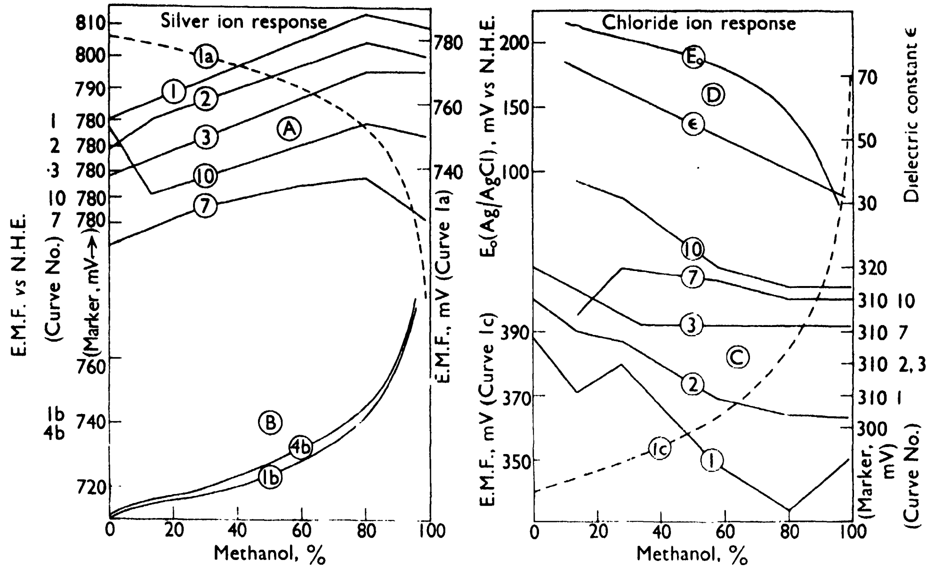

Fig. 1. Effect of ratio of solvent to water on electrode response.

Group A: electrode potentials in a solution of x ml of 10^{-1} M silver nitrate diluted to 100 ml with methanol. Curve 1a shows the response in aqueous solution of equivalent concentration

Group B: electrode potential at constant formal silver ion concentration of 10^{-3} M; curve 4b is for the silver - silver bromide electrode

Group C: electrode potentials in a solution of x ml of 10^{-1} M potassium chloride diluted to 100 ml with methanol. Curve 1c shows the response in aqueous solution of equivalent concentration

Group D: E_0, normal potential of the silver - silver chloride electrode plotted on a per cent. w/w scale; ϵ, dielectric constant of methanol - water mixture plotted on a per cent. w/w scale

Curves numbered as in Table II

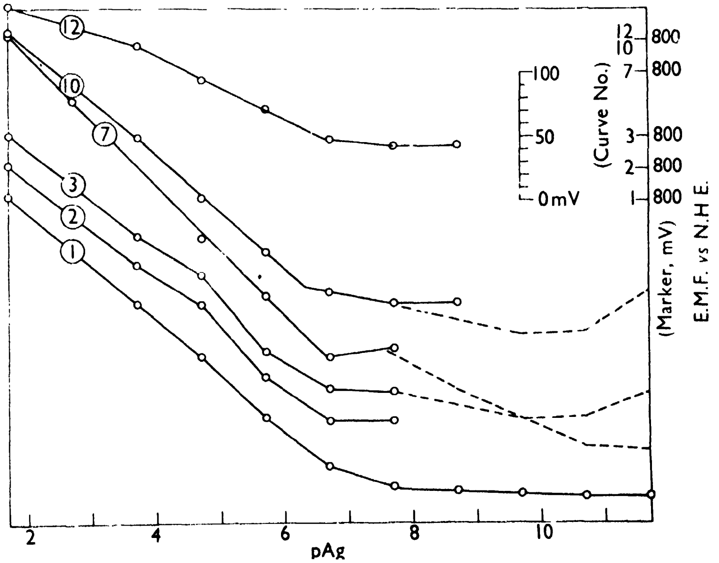

Fig. 2. Response to silver ion in 80 + 20 (by volume) methanol - water. Curves numbered as in Table II. Dotted extension to curves 3, 7 and 10 indicate the effect of induced polarisation due to the field of currents indicated at I△ in Fig. 5

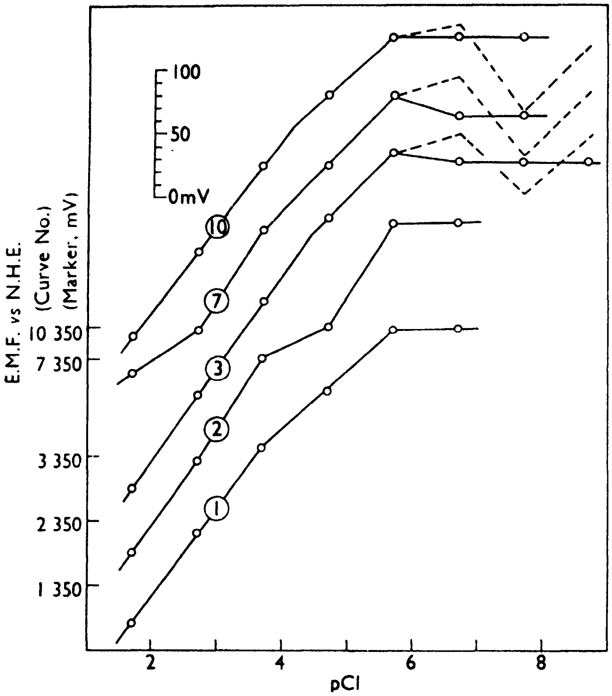

Fig. 3. Response to chloride ion in 80 + 20 (by volume) methanol - water. Curves numbered as in Table II. Dotted extensions to curves 3, 7 and 10 indicate the effect of induced polarisation by the currents indicated at 1Δ in Fig. 6

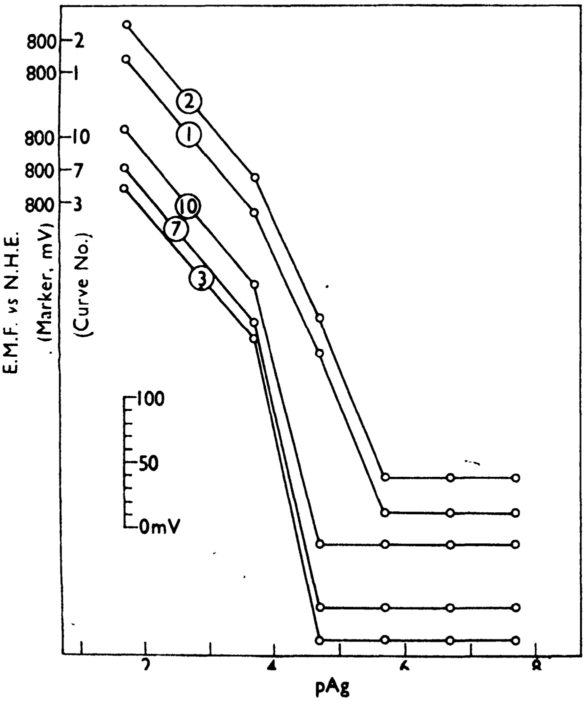

Fig. 4. Response to silver ion in 80 + 20 (by volume) acetone - water

ZERO-CURRENT ELECTRODE RESPONSE

Earlier work has been confined to standard potentials at fixed concentration of the silver chloride electrode in pure methanol,[15,16] ethanol[17] and acetonitrile.[12,18] Janz[12] reports good stability and reproducibility in the alcohols, but instability in acetonitrile, presumably because of complexation. From the foregoing results and their titrimetric performance,[3,4,19] 80 per cent. v/v methanol and acetone were chosen for extended study as a first entry into the problem, and solutions for measurement were prepared by diluting 20·00 ml of a standard aqueous solution of silver or chloride ion to 100 ml with solvent. Silver chloride being titrimetrically desirable[3,4,11] and having a reasonable durability[1] was chosen to represent halide-coated electrodes. Amalgam electrodes have not previously been examined, but have performed well in titrimetry.[4]

The silver ion response curves in 80 per cent. v/v methanol are shown in Fig. 2, and those for chloride ion in Fig. 3. Silver ion response in 80 per cent. v/v acetone is shown in Fig. 4. The critical values, following the notation of the earlier communication,[1] calculated from the curves are shown in Table II.

Potentials in these media are neither so reproducible nor so stable as in water. Reproducibility (*a*) of different specimens of the same type of electrode in a given solution and (*b*) of a given electrode transferred with intervening washing from one solution to another of the same composition, is about ±2 mV. Unless cleaning or washing of electrodes is extremely thorough, the reproducibility may fall to ±15 mV. Equilibration, especially at extreme dilution, is a little slower than in water.

TABLE II

RESPONSE OF ELECTRODES TO SILVER OR CHLORIDE ION IN SOLVENT - WATER MIXTURES AT 20° C

Electrodes	(1) Ag clean*	(2) Ag dirty†	(3) AgCl	(7) Ag/Hg aged	(10) Au/Hg aged	(12) Ag micro
Response to Ag^+ in 80 per cent. v/v methanol—						
π_0 (extrapolated), V	0·877	0·870	0·868	0·917	0·878	0·853
Initial slope, mV/pAg	42·2‡	38·8	39	—	41·8‡	15
Limit, pAg	6·7	3·7	3·7	—	6·3	3·7
Intermediate slope, mV/pAg	—	—	40§	—	—	25
Limit, pAg	—	—	6·7	—	—	6·6
Final slope, mV/pAg	16	40·7§	3	51	10	5
Failure point, pAg	7·8	6·7	7·7	6·6	7·7	7·7
Response to Cl^- in 80 per cent. v/v methanol—						
π_0 (extrapolated), V	0·205	0·197	0·203	0·252‖	0·229	—
Initial slope, mV/pCl	68‡	75·5	72·5	erratic	66·5	—
Limit, pCl	3·7	3·7	4·4	3·7	4·1	—
Final slope, mV/pCl	43§	52§	51	52‡	45	—
Failure point, pCl	5·7	5·7	5·7	5·7	5·7	—
Response to Ag^+ in 80 per cent. v/v acetone—						
π_0 (extrapolated), V	0·910	0·912	0·909	0·903	0·907	—
Initial slope, mV/pAg	59	59	57·8	59·8	59·5	—
Limit, pAg	3·7	3·7	3·7	3·7	3·7	—
Final slope, mV/pAg	115·5	115·5	232	219	200	—
Failure point, pAg	5·7	5·7	4·7	4·7	4·7	—

* Cleaned in (1 + 1) nitric acid and washed for 5 minutes with distilled water.
† Immersed in 0·01 M potassium chloride for 1 hour and washed thoroughly with distilled water.
‡ Slight deviations from linearity, ±2 mV.
§ Considerable deviations from linearity, ±10 mV.
‖ From mean slope.

Electrode behaviour in methanol differs little from that in water.

1. All electrodes respond first order to silver ion, with less than theoretical slopes. Silver amalgam again gives the best slope and response range.

2. All electrodes respond second order to chloride ion, with greater than theoretical slopes; fouled silver metal behaves similarly to silver chloride.

Generally, there is a marginal improvement in response range over water; the principal differences are described below.

3. A considerable improvement in slope, accompanied by a decrease in response range for the silver ion response of silver amalgam. A slight improvement in slope, together with some extension of range for the silver ion response of clean silver.

4. A major decrease in slope combined with an extension of response range in the silver ion response of micro (0·004 sq. cm) silver electrodes—the reverse of the size effect in aqueous media.

5. An increase of about 30 mV over the aqueous value in the overpotential, $\pi_0 - E_0 = 70$ mV, for both chloride and silver ion.

Electrode behaviour in acetone is considerably different from that in either water or methanol.

1. The overpotentials, $\pi_0 - E_0$, are considerably greater.

2. The initial slopes are greatly improved and are practically theoretical, but the response range remains limited and is the same for all electrodes.

3. At the end of the initial range, the slopes, instead of decreasing, suddenly increase by a factor of 2 for silver metal or 4 for coated electrodes, and are maintained at this very high value for a further one (coated electrodes) or two (metal electrodes) pAg units, and then the response fails abruptly, all electrodes becoming stable constant-potential generators.

This remarkable behaviour[2] accords with titrimetric evidence[3,4] that acetone is an excellent solvent for chloride determinations at titrant concentrations down to 10^{-3} M, but is useless at lower concentrations and in the determination of other halides because the electrodes are rapidly poisoned. Even higher slopes have been depicted, but not commented on, for the silver sulphide system.[20] The response curves indicate a profound change in the energetics of the electrode surface layers, and an explanation may lie in the shape of the organic molecule and the adsorption of solvent molecules within the pores of the electrode surface; this would also account for the difficulty in re-activating electrodes poisoned by such materials.

The observations recorded so far confirm the difficulty of generalising on the effect of organic solvents, even within a homologous series, or of enunciating guiding rules. The effect of each solvent is specific and must be individually investigated.

EFFECT OF RESTORING CURRENTS ON ELECTRODE RESPONSE

Zero-current electrode response fails, even in solvent media, at active ion concentrations below 10^{-5} to 10^{-7} M. The titrimetrically beneficial increase in silver halide formation constant in solvent media is not accompanied by any significant extension of electrode response range. Consequently, halide determinations at titrant concentrations below about $10^3 S^{\frac{1}{2}}_{AgX}$ will run through a significant region around equivalence, where the zero-current indicator electrode is unpoised except by chance of induced polarisation (*vide infra*) whether in aqueous or solvent media; at higher titrant concentrations this region is traversed by so small a titrant increment as to be unimportant. At titrant concentrations of $10^2 S^{\frac{1}{2}}_{AgX}$ the zero-current potentiometric method fails entirely.[3] Nevertheless, micro volumes of 10^{-9} M bromide and iodide and 10^{-6} M chloride and macro volumes at slightly higher concentration have been titrated volumetrically with silver solutions of commensurate concentration by DEP,[3,4] and even 10^{-11} mole of chloride at a concentration of $2{\cdot}4 \times 10^{-8}$ M has been titrated coulometrically by kinetic response DEP.[3,6] At extreme dilution the polarising current does more than merely differentiate.[2,3,4] The potential of an electrode polarised by a fixed and not too large current depends on the bulk concentration of the active ion, is highly reproducible, is diffusion controlled[5] and responds rapidly to change in polarising current.[21] Stable reproducible potentials can therefore be expected from electrodes carrying appropriate heavily stabilised currents. Because all the work at extreme dilution[2,3] has been done in solvent media, and in view of the poisoning effect of acetone, attention has been confined to 80 + 20 v/v methanol - water solution and information sought on the behaviour of both macro[4] and micro[3] electrodes in respect to silver and chloride ion response.

The electrode response at zero current was taken by the progressive dilution method[1] until the failure point was reached. From this point on a polarising current was applied,[10,11] anodically for chloride ion response and cathodically for silver ion response, a second silver electrode immersed in the same solution being used as the counter electrode. The polarising current was gradually incrementally increased from zero, until the stable electrode potential fell on a straight line or the extrapolation of the zero-current response curve. The equilibration speed was high compared with zero-current potentials; even at pAg = 11, the potentials reached equilibrium within 30 minutes, and thereafter varied by not more than ±0·1 mV over a period of many hours.

The results for silver ion response are shown in Fig. 5, group (A) being for micro electrodes and group (B) for macro electrodes. The cathodic curves (e) indicate that linear response has been restored to pAg = 10·7 for micro and pAg = 11·7 for macro electrodes. Beyond these points the potentials begin to oscillate slightly. The zero-current response curves (c) are included for comparison, and the improvement in range is evident. The restoring current I_Δ rises to a maximum at pAg = 9·7 and thereafter declines; for macro electrodes the I_Δ returns to zero.

Failure of zero-current electrode response is probably due to the energy barrier presented by the double layer on the electrode surface, the ionic population at low concentration being unable to maintain an adequate exchange current. Polarographically, capacitance charging current of the DME becomes equal to the faradaic current at about 10^{-5} M, and Hickling and Taylor[22] have identified the major function of a polarising current on the silver ion response of a silver electrode with the charging of the double-layer capacitor. The restoring current may be expected to fulfil this function. Freyberger and de Bruyn[23] have shown that the differential capacitance of the silver iodide electrode is a function of the silver ion concentration and reaches a maximum at pAg = 7 to 9 depending on the ionic strength, μ, of the solution. The present value of pAg = 9·7 in the absence of supporting electrolyte and μ equal to the ion product of the solvent are in good agreement. The reason for the increased current density demand of micro over macro electrodes is at present obscure, but is probably connected with the low slope, and may relate to mechanical stress in the metal.

In Fig. 6, the anodic curve (2) (*a*) indicates that the chloride ion response has been restored compared with the zero-current curves (2) or (3) in Fig. 3. The termination at pCl = 8·7 in a pure halide medium is set by the behaviour of the cathode, curve (1) (*e*), and would not necessarily apply in a solution containing silver ion. The restoring current demand shown on a logarithmic scale as (I_Δ) is much higher than for silver ion, which confirms the deduction[3] that, at moderate current densities, the DEP anode is a constant-potential

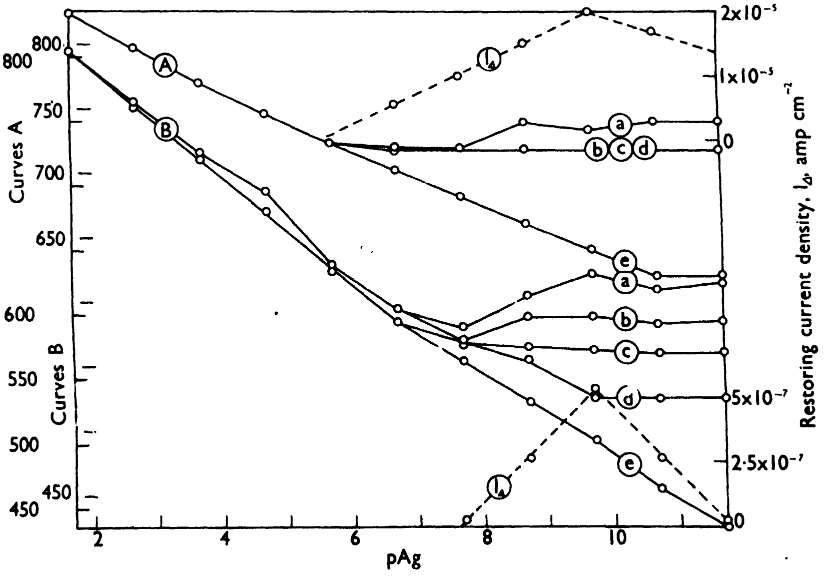

Fig. 5. Effect of restoring currents on the response to silver ion in 80 + 20 (by volume) methanol - water. (A) micro electrodes (0·004 sq. cm); (B) macro electrodes. Curves a, anode potential; b, rest potential of anode after switching off current; c, zero-current response; d, rest potential of cathode after switching off current; e, cathode potential; I_Δ, linear plot of restoring current density for curves a and e

generator leading to Z-shaped differential curves, but chloride ion response is restored at very high current densities leading to the normal peak form of differential curve.

Because the restoring function demand on the polarising current varies with concentration at low concentrations, it follows that the residue of the current that serves for differentiation in DEP will vary if the total current is kept constant, and so will give rise to the observed aberrations in anodic, cathodic and differential titration curves.[3,4] It also follows that, although what has previously been said about the fundamental unsoundness of potentiometric measurements with singly polarised electrodes at customary concentrations remains true,[5] such electrodes with appropriate control of the current can be useful in the hitherto unexplored regions beyond the failure limit of zero-current electrodes.

OTHER EFFECTS OF CURRENTS AND THEIR FIELDS

COUNTER POLARISATION—

A working polarised electrode implies a counter electrode either in the same solution, as here and in DEP, or isolated in a separate compartment. In the cathodic silver ion response measurements in Fig. 5, the counter anode was a fouled silver electrode whose response is given in the curves (*a*). The counter anode assumes a potential somewhat positive to the zero-current electrode (*c*) becoming a constant-potential electrode at high pAg values. The reaction is stripping of silver from the surface leading to a slightly enhanced silver ion concentration on the electrode surface. In the anodic chloride response measurements of Fig. 3, the counter cathode was a silver electrode (*e*) whose potential ran strongly negative in the absence of any depositable metal ion, leading to a parasitic discharge of solvent ion, *i.e.*, hydrogen ion. In DEP titrimetry silver ion would be present to prevent this.

PRE-POLARISATION—

Anodic or cathodic pre-treatment of electrodes improves their titrimetric performance[24,25] and is effective in cleaning.[26] Pre-cathodisation of a silver electrode, followed by zero-current response measurement, showed an extension of linear response range by one pAg unit, but at pAg > 7 continuous passage of current is necessary.

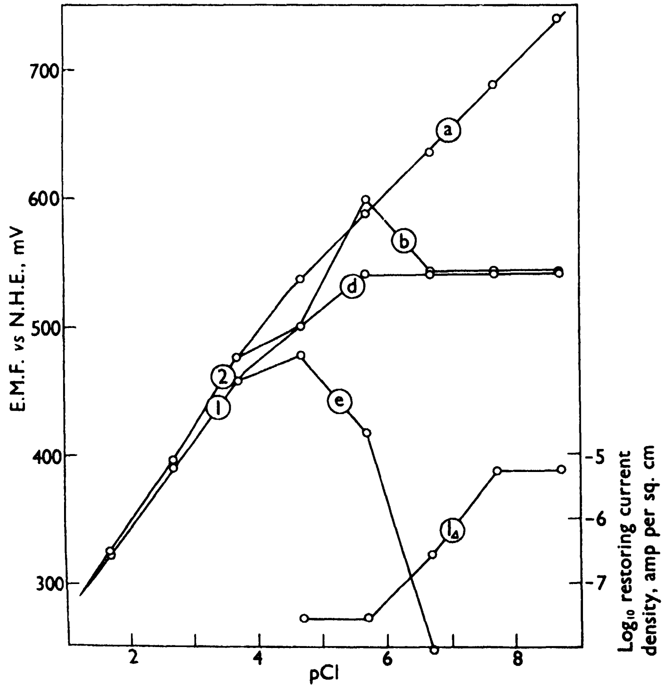

Fig 6. Effect of restoring currents on chloride ion response in 80 + 20 (by volume) methanol - water. Curves labelled as in Figs. 3 and 5; IΔ is a logarithmic plot

TIME-DECAY POLARISATION—

At concentrations below the zero-current failure point, if the polarising current is stopped the potentials drift for a short time, coming to rest at a value intermediate between the polarised and zero-current values, as illustrated by the anodic time-decay curves (*b*) and cathodic time-decay curves (*d*) in Figs. 5 and 6. In the extreme, the rest potential is the same as the zero-current potential, as with micro electrodes. The drift is in a direction of an apparent increase in the indicated ion concentration, and freshly prepared or activated electrodes show this effect more markedly. Similar results have been recorded for the hydrogen ion response of platinised platinum electrodes.[27]

INDUCED POLARISATION—

Zero-current electrodes immersed in a solution containing other electrodes through which current is passing display active response beyond the normal failure point, as illustrated by the broken line extensions to curves (3), (7) and (10) in Figs. 2 and 3. Initially, this response is in the proper direction, but, as the ionic concentration decreases, aberrations occur and the response becomes spurious. When the polarising current is stopped, normal zero-current response is restored. Such polarisation induced by fields from current-carrying electrodes explains certain phenomena observed in titrations at low concentration.[4]

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Silver- and Halide-ion Responsive Electrodes

Part III. Ionic and Electron Transfer Interaction and the Effect of Supporting Electrolytes

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The interactions of cations and electron transfer systems on the silver ion response of BH68 membrane electrodes, of diverse halide ions on the halide response and of electron transfer systems on the silver- and halide-ion responses of metal, halidised and amalgam electrodes have been examined and tolerance limits established. Supporting electrolytes improve response slopes, but shorten response ranges of the electrodes, and saturation of the solutions with silver halide has no effect on the halide-ion response characteristics.

EARLIER papers[1,2,3] reported the preparation and handling of electrodes, their zero-current response in aqueous and mixed solvent - water media free from supporting or other electrolytes, and the restoring effect of polarising currents on linear response. This has placed on a quantitative basis many of the speculative explanations of phenomena encountered in potentiometric and differential electrolytic potentiometric (DEP) argentimetric titrimetry,[4,5] but several questions remain unanswered, and are posed below.

Experimental details have been given.[1] The general method of determining the tolerance limit for a potentially interfering ion of the electrode response to an indicated ion was to prepare solutions of a constant indicated ion concentration containing increasing concentrations of the interfering ion and to measure the potential of the proper zero-current indicator electrode in the solutions. The solution for which the electrode potential deviated by ±1 mV from the expected value,[1] *i.e.*, a ±4 per cent. alteration in the indicated concentration, contained the maximum tolerable concentration of the interfering ion.

INTERACTION OF CATIONS ON THE SILVER ION RESPONSE OF BH68 GLASS MEMBRANE ELECTRODES

Electron-transfer systems interfere seriously with potentiometric argentimetry,[6] and, since glass is indifferent to redox systems, it was hoped that the new cation responsive glasses would provide a silver responsive electrode free from this defect. Although particular responses can be emphasised,[7] these membrane electrodes respond to most univalent cations, and the tolerance of the silver response for such ions had to be determined. The speed, range and specificity of response of BH68 membrane electrodes (kindly supplied by Electronic Instruments Ltd., Richmond, Surrey, as GNA23 electrodes) improves considerably on ageing in a solution of the selected ion.[1,5] The response of such electrodes to a single ion of activity *a* is reported[8] to accord with—

$$E = E_0 + \frac{2{\cdot}303\,RT}{F}\log_{10}a$$

and after conditioning for 8 days in 0·1 M silver nitrate the linear response to silver ion was found[1] to extend to pAg = 5, with kinetic[1] or instantaneous response down to pAg = 7, though the equilibrium potentials deviated from linearity at pAg > 5. In the presence of other univalent cations, *e.g.*, sodium, the mixed response is reported[8] as—

$$E = E_0 + \frac{2{\cdot}303\,RT}{F}\log_{10}\left(a_{\mathrm{Ag^+}}^{\frac{1}{K_1}} + K_2\,a_{\mathrm{Na^+}}^{\frac{1}{K_1}}\right)^{K_1}$$

where K_1 and K_2 are constants for the particular glass. The conditioned electrode showed the tolerances listed in Table I. The hydrogen ion deviation is not serious at pH = 3, but takes control at pH = 2. Increase in potassium ion concentration gives first an increased

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TABLE I

TOLERANCE FOR UNIVALENT CATIONS OF THE SILVER ION RESPONSE OF BH68 GLASS MEMBRANE ELECTRODES CONDITIONED FOR 8 DAYS IN 0·1 M SILVER NITRATE

Indicated ion concentration [Ag^+]	Interfering ion	Tolerable concentration of interfering ion, M
0·1	K^+	0·07
0·1	Na^+	0·01
0·1	H^+	10^{-4}

and then a constant potential, whereas the potential increases continuously with increase in sodium ion concentration. Halide ions are without effect, even on a conditioned electrode, which merely takes up the potential displayed in water or in silver ion at $< 10^{-7}$ M.

Iron^{III} ion is tolerated up to one-fifth of the silver ion concentration, but in a 5×10^{-2} M silver solution iron^{II} ion concentrations of 5×10^{-2}, 5×10^{-3} and 5×10^{-4} M caused potential deviations of +80, +36 and −5 mV, respectively, thus confounding the hope expressed above; a conclusion confirmed by titrimetric experience.[5] The iron^{II} ion concentration must not exceed one-hundredth of the silver ion concentration. The reason for this is obscure. No chemical reaction between iron^{II} and silver ion could be discerned, nor was any change in the nature of or deposition of metallic film upon the electrode surface detectable.

INTERACTION OF HALIDE IONS

Pinching and Bates[9] showed that 0·01 mole per cent. of bromide alters the potential of a standard silver chloride electrode by 0·1 to 0·2 mV. Bates[10] makes the further surprising statement that the potentials of the chloride electrode are not greatly affected by traces of iodide or cyanide. Tremblay[11] found that the bromide response of silver metal electrodes failed in the presence of chloride at an equal concentration. We have found[1] that silver bromide and silver iodide electrodes respond to chloride ion better than do silver chloride electrodes, silver iodide electrodes respond to bromide ion better than do silver bromide electrodes, silver chloride electrodes respond feebly or not at all to bromide or iodide ions and silver bromide electrodes behave similarly to iodide ion. Use has been made of these facts in increasing the selectivity and sensitivity of halide titrations at extreme dilution by DEP.[4] Gaps remain in the quantitative knowledge of the interaction of halide ions on halide response. Interactions on chloride and bromide response are shown in Table II. Chloride and bromide ion do not interfere with iodide ion response, even at a ratio of $[Cl^-]/[I^-]$ of 10^7. The chloride response of all electrodes fails in the presence of 0·1 mole per cent. of bromide, but amalgam electrodes show a better tolerance to iodide than do silver or silver halide electrodes, for which, contrary to the previous statement,[10] less than 1 part of iodide in 10^7 parts of chloride seriously interferes. An equal or greater chloride concentration is tolerated in bromide ion response, but 0·01 mole per cent. of iodide interferes; amalgam electrodes again show a favourable performance. The tolerance in the response of one ion to an interfering ion is qualitatively in the order of the solubility of the silver halides. Determination of a halide in a mixture is best performed with either an amalgam electrode or a silver electrode coated with the required halide.[4,5]

TABLE II

INTERFERENCE OF BROMIDE AND IODIDE ION WITH CHLORIDE ION RESPONSE AND OF CHLORIDE AND IODIDE ION WITH BROMIDE RESPONSE

The maximum tolerable concentration of the interfering ion is given

	Indicated ion Interfering ion	10^{-2} M Cl^- Br^-, M	10^{-1} M Cl^- I^-, M	10^{-3} M Br^- Cl^-, M	10^{-3} M Br^- I^-, M
Electrode					
Silver		10^{-5}	$<10^{-8}$	10^{-3}	10^{-7}
Silver chloride		10^{-5}	$<10^{-8}$	erratic	erratic
Silver bromide		10^{-5}	$<10^{-8}$	10^{-2}	10^{-7}
Silver amalgam		10^{-5}	10^{-6}	10^{-2}	10^{-6}
Gold amalgam		10^{-5}	10^{-6}	10^{-3}	10^{-6}

INTERFERENCE FROM ELECTRON-TRANSFER PROCESSES

Redox systems such as $Cr^{VI/III}$, $Fe^{III/II}$ and quinhydrone completely upset potentiometric halide determinations, but the process is obscure as the effect is due to the oxidant with chromium, and the reductant with iron.[6] One system was chosen to examine its effect on electrode response. $Iron^{III}$, *pace* oxidation of iodide, had little or no influence on the electrode potentials, but $iron^{II}$ caused serious disturbances, increasing in intensity for the responses in the order iodide, bromide, chloride and silver ion, as shown in Table III.

TABLE III

THE MAXIMUM TOLERABLE CONCENTRATION OF $IRON^{II}$ ION IN ELECTRODE RESPONSE TO 10^{-3} M SILVER AND HALIDE ION

Indicated ion (10^{-3} M) .. Electrode	Ag^+	Cl^-	Br^-	I^-
Silver	10^{-}	10^{-4}	10^{-}	10^{-3}
Silver chloride	10^{-}	10^{-5}	10^{-}	10^{-3}
Silver bromide	10^{-}	10^{-5}	10	10^{-3}
Silver amalgam	10^{-}	10^{-5}	10^{-}	10^{-3}
Gold amalgam	10	10^{-4}	10^{-}	10^{-3}

No chemical reaction could be detected, the difference between amalgam and other electrodes is slight, and the effect is hardly changed by alteration of the electron transfer potential by addition of $iron^{III}$. Electron response of the electrodes is possible, but improbable, and membrane electrodes show a similar behaviour; complexation is equally unlikely.

THE EFFECT OF SUPPORTING ELECTROLYTES

Most earlier work, particularly that directed towards constant or standard potentials, has been done at constant, usually fairly high, ionic strength. Titration media in the equivalence-point region have a much higher ionic strength of counter ions than of active indicated ions. Supporting electrolytes have proved effective in reducing double-layer charging currents and charge transfer overpotentials[4] at extreme dilution. It has been suggested[12] that the low slope factor of membrane electrodes[1] is due to the lack of background electrolyte. Many responses were therefore re-determined at constant supporting electrolyte concentration. Electrolytes of a suitably low halide content were difficult to find. Microtitration by DEP has shown the presence of, *e.g.*, 7 p.p.m. of bromide in potassium chloride, 2 p.p.m. of chloride in uranyl nitrate, 1 p.p.m. of chloride in perchloric acid and 15 p.p.m. of chloride, 22 p.p.m. of bromide and 63 p.p.m. of iodide in barium nitrate, all reagents of the highest grade. Transistor-grade nitric acid (kindly supplied by British Drug Houses Ltd.) proved to have a halide content of less than 10^{-11} M, and since hydrogen ion at a concentration less than 0·15 M has proved to be without effect on the electrodes, this reagent was used to give a constant concentration of 0·1 M. Nitric acid is obviously unsuitable for pH sensitive glass membrane electrodes, but these are inert to halide ion, so a trace of halide impurity insufficient to affect the silver ion concentration within the response range is tolerable, and a background of 0·1 and 0·01 M potassium nitrate[7] was used for these electrodes.

Generally, the effect of supporting electrolyte on response slopes was beneficial. Silver ion response slopes increased from about 40 to 56 mV per unit of pAg for both membrane and metal electrodes. Chloride ion response slopes were reduced to 60 mV per unit of pCl in the range pCl = 1 to 2, and to 52 mV per unit of pCl in the range pCl = 2 to 3. However, at concentrations below 10^{-3} to 10^{-4} M, the slopes rapidly tailed off. The failure points generally fell by 1 p unit, so that the response range was considerably shortened. For silver ion response, the potential span was much the same with and without supporting electrolyte, but was covered in a shorter response range in the presence of supporting electrolyte. The use of supporting electrolyte is therefore of benefit at moderate concentrations, $> 10^{-4}$ M, but is deleterious at lower concentrations.

The halide response curves[1,2,3] were taken in solutions free from silver ion. This may lead to a diffusion-controlled electrode imbalance, which may affect the potentials. This possibility was examined by repeating a selection of the determinations on solutions presaturated with silver halide. There was no detectable difference in either slope or response range under these conditions.

OTHER INFLUENCES ON ELECTRODE POTENTIAL

The rate of stirring has a profound effect on potentials in argentimetric titrimetry when a suspension of precipitate is present[6] and on DEP titrimetry, which is a diffusion controlled process.[13] In response measurements in the absence of suspended matter, the effect is relatively small, is greater for halide ion than for silver ion response, and increases with decreasing ion concentration. Potentials are always higher in unstirred solutions. The difference in potential between quiescent solutions and solutions stirred at cavitation speed for $pAg = 1$ is 1 mV, for $pCl = 1$ is 7 mV, for $pAg = 7$ is 4 to 5 mV and for $pCl = 7$ is 8 to 9 mV. Above a certain critical, fairly low, speed the potentials become independent of stirring rate. Exposure to light changes the colour of the film on halide coated electrodes, but has little effect on the potentials at concentrations above 10^{-3} to 10^{-4} M. At low concentrations the effect becomes considerable.

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The Absorptiometric Determination of Silicon in Water

Part III*. Method for determining the Total Silicon Content

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A method is described for determining the total silicon content of water at low concentrations. It consists in evaporation and subsequent fusion of samples with sodium carbonate; the resulting sodium silicate is determined absorptiometrically as reduced α-molybdosilicic acid. The absorptiometric procedure is precise, and the major errors, at low concentrations, arise in the fusion procedure. The standard deviation, for samples between 2 and 20 ml, was about 0·3 μg of silica over the range 0 to 60 μg of silica. Four samples can be analysed in duplicate per day.

A METHOD was required for determining total silicon in the make-up water, feed-water, condensate and condensed steam (0·002 to 0·2 p.p.m. of silica), and the raw water and partially purified raw water (up to 20 or 30 p.p.m. of silica) of power stations. The results were also needed for calculating the "non-reactive" silicon content of samples by subtracting their "reactive"† from their total silicon contents. As this difference may sometimes be small compared with the analytical results, the greatest possible precision was required.

A fusion technique was considered necessary to convert into sodium silicate any form of silicon that might occur in samples; fusion with sodium carbonate was chosen as being convenient and suitable. Absorptiometric determination of the silicate was chosen because of the sensitivity of this technique. Suitable conditions for determining sodium silicate absorptiometrically were reported in Part I,[2] and precise results have been obtained with a method based on reduced β-molybdosilicic acid.[1] However, the sensitivity of such methods is decreased by large amounts of salts, and the effect of neutralised sodium carbonate on the method was therefore determined; 0·5 g of sodium carbonate was used, as this was considered the minimum desirable for use with 30-ml platinum crucibles. The results of five determinations were erratically lower (by 0·3 to 4·3 per cent.) than expected in the absence of sodium salts.

The proposed method is therefore based on reduced α-molybdosilicic acid, which is not affected by large amounts of salts.[3] From the experimental results in Part I, a suitable procedure was chosen; it is described below, together with the tests made of it.

EXPERIMENTAL

APPARATUS, REAGENTS AND TECHNIQUE—

The procedure given under "Method," p. 449, was used for the work described in this section, except when indicated otherwise. Optical-density measurements were made in 4-cm cuvettes at 742 mμ with a Hilger Uvispek spectrophotometer, distilled water being used in the reference cuvette. The temperature of the laboratory varied between 18° and 25° C during the work.

Dilute suspensions of clay in water were prepared by mixing about 60 g of finely ground bentonite clay with one litre of water, and then repeatedly spinning in a centrifuge at about 2200 r.p.m., and decanting the suspensions. Suspension No. 1, prepared in this manner, had no visible turbidity, whereas suspension No. 2 was slightly turbid. Other suspensions were prepared by dilution of these with water.

For all the statistical significance tests applied in this work, the 95 per cent. confidence level was used.

Before determinations of total silicon were carried out, all surfaces in the working area and its immediate vicinity were wiped with a wet cloth to remove dust. The laboratory was not air-conditioned.

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* For details of previous parts of this series, see reference list, p. 455.

† "Reactive" silicon—mainly monomeric and dimeric silicic acid—is defined in this paper as those forms of silicon that react with ammonium molybdate in 10 minutes to form molybdosilicic acid under the conditions of the method given in Part II of this series.[1]

VARIATION IN CONCENTRATION OF REAGENTS—

The effect of variations in the volumes of ammonium molybdate, 48·5 per cent. sulphuric acid and reducing agent solutions dispensed was determined by using the same experimental design and analysis as in the analogous experiment in Part II.[1] The concentration of silicon in the final solution was 0·5 p.p.m. of silica. The results showed that a ±20 per cent. change in the concentration of ammonium molybdate or sulphuric acid gave a ±0·2 per cent. change in optical density, and that a ±20 per cent. change in the concentration of reducing agent had no significant effect compared with a coefficient of variation of ±0·24 per cent.

EFFECT OF OTHER SUBSTANCES—

Except when otherwise stated, the evaporation and fusion stages were omitted and the solutions of the substances were treated as described in the procedure for calibrating samples or reagent blank solutions. Duplicate analyses were made for each condition tested, and in each batch blank solutions and silicate solutions containing no added substance were also treated. Table I shows the effect of other substances. The effects of phosphate and chloride, which both interfere, have been described previously.[2]

Table I shows that both ionic forms of platinum interfere. However, during a fusion platinum crucibles lost only 0·1 to 0·2 mg of platinum, equivalent to 1 to 2 p.p.m. of platinum in the final solution; the effects are therefore negligible. The effects of cupric copper and nickel are probably caused by the colour of their ions in acid solutions; both substances

TABLE I

EFFECT OF OTHER SUBSTANCES

All concentrations are in the final solution

Substance	Concentration of substance, p.p.m.	Difference, silicon found less silicon added (as p.p.m. of silica), at silicon concentrations of—	
		0 p.p.m. of silica	0·5 p.p.m. of silica
Platinous	10	0·001	0·002
	20	0·003	0·004
Platinic	10	0·002	0·003
	50	0·006	−0·001
	100	0·006	−0·023
Ferrous	10	—	0·000
	50	0·001	−0·008
Ferric	2	—	0·000
	10	0·001	−0·005
	50	—	−0·057
Cuprous	2·5	0·002	0·002
Cupric	100	0·049	0·052
Nickelous	50	0·005	0·004
	100	0·011	0·009
Chromic	10	0·000	0·000
Aluminium	10	−0·001	−0·001
Zinc	10	0·000	0·000
Magnesium	100	−0·001	0·000
Calcium	100	0·004	0·003
Molybdenum^{VI}, tungsten^{VI}, titanium^{IV}, vanadium^{V}, manganous, stannous and cobaltous	0·1 of each	0·000	0·000
Potassium	4500	0·009	0·006
Sulphate	5500	0·009	0·006
Nitrate	155	0·004	0·003
Fluoride	5	0·004	0·004
Morpholine. cyclohexylamine, octadecylamine and hydrazine sulphate*	50 of each	0·011	−0·009
Alkylarylsulphonate (detergent powder containing 42 per cent. of active material and no added phosphate or silica)*	10	0·019	0·010
0·5 g of sodium carbonate, neutralised by 10 ml of N sulphuric acid	—	0·011	0·011
Significant effects are greater than		0·002	0·003

* The entire procedure was carried out.

in 2·5 N sulphuric acid gave effects of similar magnitude to those reported in Table I. Some of the interference effects shown in Table I are possibly caused by silicon in the substance added.

FUSION PROCEDURE—

Contamination—From time to time during the development work on this method erratically high results were obtained and were traced to contamination from various sources. These are discussed below.

One of four platinum crucibles was shown to give consistently higher results than the others. This effect was little changed by bisulphite or carbonate fusions, but was reduced considerably by repeated evaporation of hydrofluoric acid from the crucible, and may have been caused by a piece of siliceous material embedded in the wall of the crucible.

Initial difficulty was also experienced from airborne contamination during the evaporation stage. However, by using the hood described under "Method," the incidence of unduly high results was reduced from 13 out of 82 to 3 out of 89. Further tests showed that the general level of airborne contamination during the evaporation stage was equivalent to less than 0·2 µg of silica.

Some contamination occurred when the sodium carbonate was being fused; the magnitude of the effect depended on where the fusion was carried out. This effect was measured by comparing the optical densities of fusion blank solutions with those of similar solutions prepared by fusing the carbonate with the crucible lids left on all the time. In a fume cupboard, this contamination was variable and averaged 1 µg of silica. On one occasion on a bench in the laboratory it averaged only 0·1 µg of silica (one result, of 1·1 µg, out of 12 was excluded). On other occasions, although it was not directly checked, it appeared to be no more than about 0·1 µg of silica. Although the effect was small when fusions were carried out on the bench, it could not be guaranteed to be absent, and therefore fusion blank values were always checked before and during a run of sample determinations; the results were then examined for evidence of contamination. Duplicate portions of samples were always analysed, and when the results differed by unusually large amounts, further determinations were done and the discordant values rejected.

Silicon in the sodium carbonate—Various methods of reducing and homogenising the silicon content of AnalaR sodium carbonate were tested, the most effective being simply to filter a solution of the carbonate and then evaporate to a powder; details are given under "Method." Millipore filters, with pore sizes of 450 and 10 mµ, were no more effective than Whatman No. 542 filter-papers in reducing the silicon content of the carbonate. Table II shows the fusion blank values (see "Method," p. 449) obtained with sodium carbonate produced in this maner and also with various commercially available grades.

TABLE II

FUSION BLANK VALUES FOR VARIOUS GRADES OF SODIUM CARBONATE

Sodium carbonate	Number of determinations	Number of unduly high results, excluded from calculations	Remaining results	
			Mean, µg of silica	Standard deviation, µg of silica
AnalaR, batch 1, mixed mechanically	17	4	4·8	5·1
Specpure, batch 1, not mixed	3	0	8·4	1·5
Micro-analytical reagent:				
B.D.H. batch 1, not mixed	9	1	2·4	0·21
B.D.H. batch 2, not mixed	9	0	0·8	0·25
B.D.H. batch 2, mixed mechanically	18	1	0·8	0·10
H. & W., batch 1, not mixed	9	3	3·6	0·25
Specially purified (see Method):				
batch 1	9	0	1·7	0·21
batch 2	18	0	2·8	0·25

Effect of duration and temperature of fusion—The procedure described under "Method" (*i.e.*, fusion at bright red heat for 6 minutes) was compared with what should be the worst conditions for (*a*) incomplete fusion and (*b*) losses by vaporisation. Fusion for 3 minutes—the shortest time in which it was possible to rotate the crucible so that the sodium carbonate came into contact with the entire inner surface—at the lowest temperature for just melting sodium carbonate, gave results for clay suspensions 0·5 per cent. lower than those by the normal procedure; this difference was not significant. Fusion for 18 minutes with three Meker-type burners using town-mains gas to heat each crucible gave results for sodium silicate solutions 0·6 per cent. higher than those for the normal procedure; this difference was not significant. Therefore, within the ranges likely with the apparatus and procedure specified, any effects caused by the temperature and duration of fusion were less than 1 per cent.

Effect of order of dissolving and neutralising the melt—Two alternative procedures were compared with the procedure described under "Method" (*i.e.*, addition of a solution of the melt to excess of sulphuric acid). Addition of sulphuric acid to a solution of the melt from a clay suspension gave results 0·6 per cent. higher than those by the normal procedure; this increase was not significant. However, dissolution of the melt in sulphuric acid and dilution with water gave a 2·6 per cent. increase with a clay suspension and a 0·6 per cent. increase with a sodium silicate solution. The limits for detecting a significant difference were 1·9 per cent. and 1·2 per cent., respectively; the result for the clay suspension is, therefore, statistically significant. The procedure described under "Method" was adopted in spite of these figures, because the effect was small and it is more difficult to avoid losses and contamination when sulphuric acid was added to the melt.

METHOD

APPARATUS—

Evaporating hood—An inverted large glass funnel, placed about 2 mm above the surface of the hot-plate, forms a suitable hood. Pass a gentle stream of air (about 500 ml per minute) filtered through two Whatman No. 542 filter-papers (or suitable alternative filter) down the stem of the funnel so that condensation does not take place on the inside of the funnel.

Platinum crucibles—Thirty-millilitre platinum crucibles are suitable. A record of fusion blank values from each crucible will show whether any particular crucible gives results consistently different from the others.

Polythene bottles—Eight-ounce narrow-mouthed polythene bottles are used in the colorimetric stage. As some bottles have initially given systematically high results, treat all bottles as described below before first using them. Allow 20 ml of 2·8 per cent. sulphuric acid to stand in each bottle for 30 minutes, then add 10 ml of ammonium molybdate solution and 100 ml of distilled water, and heat for 1 hour in a boiling-water bath. Discard the solutions, rinse the bottles well with distilled water, and then test them as described below. Allow 10 ml of 2·8 per cent. sulphuric acid to stand in each bottle for 30 minutes, and then add 5 ml of ammonium molybdate solution, and treat the solutions further as described in the procedure for the colorimetric stage. Use these optical densities to detect the bottles giving high results. Further cleaning of these particular bottles may make them satisfactory.

REAGENTS—

All reagents should be of analytical-reagent quality unless otherwise stated. Only silicon in the reagents added before the 48·5 per cent. sulphuric acid solution contributes to the reagent blank value; therefore polythene-ware or well-rinsed glassware is preferably used in their preparation. Reagents should be stored in polythene bottles.

If techniques are chosen so that the maximum error involved in each of (*a*) weighing out chemicals, (*b*) adjusting reagent solutions to volume and (*c*) adding the required volume of reagent solution to samples, is less than 5 per cent. (except when other limits are specifically stated), errors from these sources should be small.

Water—Distilled water from a Manesty still, stored in polythene, was found suitable and usually contained less than 0·005 p.p.m. of silica.

If the water used for dissolving the fusion melt contains silicon, then in general the variations in the amounts of water used will cause errors, the magnitude of which may be estimated from the "reactive" silicon content of the water. The high-sensitivity modification of the method for "reactive" silicon described previously[1] is suitable for determining the silicon content. The total silicon content of the water may be higher than the "reactive" silicon content and for some purposes, *e.g.*, careful checks for bias, should be determined by the method, and the appropriate corrections made.

Sodium carbonate—Determine the homogeneity of the material by fusing about ten 0·5-g portions for a few minutes with the lids left on the crucibles, treating them further by the normal procedure and calculating the standard deviation and mean silica content of the final solution. Also obtain an estimate of the within-batch standard deviation of the reagent blank value, and use all these figures to decide whether or not the material gives adequate precision.

Some batches of M.A.R. sodium carbonate (obtained from British Drug Houses Ltd.) were suitable for our purposes after being mixed mechanically for several hours.

If a suitable commercial product is not available, prepare one as described below. Dissolve 200 g of analytical-reagent grade sodium carbonate, Na_2CO_3, in 1000 ml of water in a polythene beaker; use a polythene stirring rod. Filter this solution through a Whatman No. 542 filter-paper held in a polythene funnel, and collect the filtrate in a polythene beaker; reject the first 50 ml, and keep the beaker well covered throughout. Evaporate this solution to dryness in batches; use platinum dishes (100 ml or larger) that have been cleaned by sodium carbonate fusion, and maintain a cover about 5 cm above the dishes during the evaporation. As the carbonate crystallises, break up large lumps with a spatula before they become dry and solid, as they are difficult to grind. Grind the material to a fine powder in a metal container with a metal rod; polythene was once found to cause contamination. Mix the powder well, by mechanical means if possible, and store in a polythene bottle. Test as described above.

Ammonium molybdate solution—A 5 per cent. w/v solution of ammonium molybdate tetrahydrate in water. A white deposit may appear on the container walls, but the reagent was usable for at least 2 months.

Sulphuric acid, 48·5 *per cent. v/v, aqueous.*

Sulphuric acid, 2·8 *per cent. v/v*—Dilute with caution 28 ± 0·5 ml of concentrated sulphuric acid to 1 litre with water. Prepare sufficient to last for an entire batch of sodium carbonate, otherwise the fusion blank value (see later) may alter.

Sulphuric acid, 0·14 *per cent. v/v, aqueous*—Prepare sufficient to last for an entire batch of sodium carbonate.

Stannous chloride solution—Prepare not more than 3 hours before use. Allow a clean stoppered 50-ml cylinder or flask to drain thoroughly (about 60 seconds), place in it a 0·1-g piece of tin foil (obtainable as 0·1-g pieces from the British Drug Houses Ltd.), add 1·5 ± 0·3 ml of concentrated hydrochloric acid, and allow to dissolve. Small black particles left in the solution may be ignored if no bubbles of gas are evolved from them. Dilute to 50 ml with water.

Standard solutions of silicon—Fuse 1·000 g of pure dry silica with 5 g of analytical-reagent grade anhydrous sodium carbonate at red heat in a platinum crucible; dissolve the cooled residue in water, and dilute to exactly one litre. This solution contains 1000 p.p.m. of silica. Prepare, by dilution, a solution containing 10 p.p.m. of silica. The solutions containing 1000 p.p.m. were stable within ±0·5 per cent. for at least 2 years in polythene bottles, and the solutions containing 10 p.p.m. for at least 3 months.

PROCEDURE—

Size of sample—The selection of sample size is important in this method in order to obtain the highest precision. A balance should be struck between: (*a*) a large error contribution from the fusion blank with small volumes and (*b*) increased evaporation times with large volumes (at low temperatures) or increased spectrophotometric errors with high optical densities (at high concentrations). In our work, 20-ml samples were the largest used. Samples larger than 10 ml should be evaporated in 10-ml portions.

Evaporation and fusion stage—Clean the platinum crucibles by fusing in them about 1 g of analytical-reagent grade sodium carbonate, rotating each crucible so that the melt touches the entire inner surface. Allow to cool, and wash out the melt. (Omit the fusions if the previous determinations in the crucibles were by this method.) Rinse the crucibles well inside and outside, and dry the outside with clean filter-paper; do not dry the inside.

By pipette, place a suitable portion of sample (not more than 10 ml) in a clean crucible, and evaporate to dryness on a hot-plate under a hood. The hot-plate should be as hot as possible short of causing the sample to boil or spit. If the required volume of sample was more than 10 ml, evaporate further 10-ml portions in the same crucible.

With the crucible being kept covered whenever possible, add 0·5 ± 0·002 g of purified sodium carbonate, and fuse by heating to bright red heat over a burner. Rotate the crucible in the flame so that the melt touches the entire inner surface, except where the tongs are holding. Replace the lid, and heat for 1 to 2 minutes. Allow to cool, nearly fill the crucible with water from a polythene wash bottle, and heat gently to dissolve the melt rapidly without bumping or boiling.

Add 10 ± 0·2 ml of 2·8 per cent. sulphuric acid to a clean narrow-mouthed 8-oz polythene bottle; in cleaning, the walls should be well rinsed, *e.g.*, by twice quarter filling with water, and inverting. Quantitatively transfer the contents of the crucible through a polythene funnel into the bottle; pour the solution out in one continuous stream, and avoid as far as possible having it run down the outside of the crucible, where it may be contaminated. Do not tip the crucible back before the rinsing has been completed. Rinsing should be controlled so that the final volume in the bottle is 60 ± 5 ml. These solutions may be left overnight if necessary, until all the bottles for a batch have been filled.

Colorimetric stage—Put 5 ml of ammonium molybdate solution into the polythene bottle, and cover the mouth with a loose-fitting polythene cover. Heat the bottle in a boiling-water bath for 1 hour ±5 minutes. Place the bottle in cold water, allow to cool to room temperature or preferably below, add 15 ml of 48·5 per cent. sulphuric acid, noting the time, and mix. One minute ±5 seconds after the sulphuric acid has been added, add 2 ml of stannous chloride reagent, and mix. Transfer the liquid quantitatively to a 100-ml calibrated flask, place the flask in cold water, and cool to 20° ± 2° C (or some other chosen standard temperature; other limits on this temperature may be chosen to give the required precision[2]). Adjust the liquid to the mark with water.

Within one hour after adding the reducing agent, measure the optical density of the solution in 4-cm cuvettes with distilled water in the reference cuvette; keep the solutions out of direct sunlight. The best wavelength to use is 742 mμ (the peak is sharp and should be checked on each instrument), but Ilford 608 or equivalent filters can be used if necessary. Subtract the optical density of the reagent blank solution from that of the sample, and calculate the concentration of silica in the final solution from calibration results.

Calibration samples and reagent blank tests—For calibration samples, place by pipette the required volumes of standard silicon solution (7·5 ml of the solution containing 10 p.p.m. gives an optical density of about 0·95 at 742 mμ) in clean 8-oz polythene bottles, and add sufficient water to make the volumes 50 ± 5 ml; for reagent blank solutions merely place 50 ± 5 ml of water in the bottles. Add 10 ml of 0·14 per cent. sulphuric acid, and carry out the procedure of the colorimetric stage. One uniform batch of water should be used for the samples, reagent blank solutions and calibration samples in each batch.

Between-batch errors may be allowed for, if large, by including calibration samples (say 0·5 p.p.m. of silica) with each batch of samples, and using their optical densities in the calculations. Alternatively, the usual calibration curves may be used.

When adding liquids from a pipette into polythene or platinum vessels, draining the pipette with its tip held against the container walls causes relatively large errors (coefficient of variation about ±0·15 per cent.). It is preferable to place the tip of the pipette just under the surface of the liquid and to leave it there for the required draining period (coefficient of variation about ±0·015 per cent.).

Fusion blank test—Weigh out 0·5 ± 0·002 g of sodium carbonate into a clean platinum crucible, and place on a hot-plate under the hood for the same period as for samples. Carry out the rest of the procedure in the same manner as for samples; ensure that the crucibles are rotated in the flame when fusing the carbonate.

In these laboratories, the variation of the fusion blank value was more than the errors of the colorimetric determination, and therefore the fusion blank figure used in the calculation of results should be the mean of many determinations (at least about ten) on the batch of sodium carbonate used. It is advisable to carry out a further determination with every few samples as a check on the procedure and on the reagent.

Calculation of results—Subtract the mean fusion blank value (as p.p.m. of silica in the final solution) from the measured concentration of silica in the final solution, and multiply by the factor 100/sample volume, to give the concentration of silicon in the sample, expressed as p.p.m. of silica.

RESULTS

LINEARITY OF CALIBRATION CURVE—

A calibration curve prepared as described under "Method" was linear within ±0·5 per cent. to at least 1 p.p.m. of silica in the final solution. The slope, with 4-cm cuvettes at 742 mμ, was 0·790 p.p.m. of silica in the final solution per unit of optical density.

PRECISION—

Precision of the colorimetric stage—The experimental design used to estimate precision was similar to that previously described.[1] The samples were (*i*) sodium silicate solutions, analysed in batches during 2½ years and (*ii*) synthetic soft water (containing sodium silicate *plus* 15 p.p.m. of Ca^{2+}, 7 p.p.m. of Na^+, 1·5 p.p.m. of Mg^{2+}, 0·5 p.p.m. of K^+, 0·5 p.p.m. of Al^{3+}, 0·25 p.p.m. of Fe^{3+}, 0·25 p.p.m. of Cu^{2+}, 0·1 p.p.m. of Mn^{2+}, 46 p.p.m. of SO_4^{2-}, 11 p.p.m. of Cl^- and 0·8 p.p.m. of NO_3^-) analysed in batches over a period of 16 days. As 50-ml samples of synthetic soft water were used, the concentrations of the elements mentioned above in the final solution were half those stated. Table III shows the standard deviations of the differences—optical density of sample *minus* optical density of reagent blank solution expressed as p.p.m. of silica.

TABLE III

PRECISION OF COLORIMETRIC STAGE

All concentrations are in the final solution

	Sodium silicate solutions		Synthetic soft water		
	Reagent blank	0·5 p.p.m. of silica	0 p.p.m. of silica	0·1 p.p.m. of silica	0·5 p.p.m. of silica
Within-batch standard deviation, p.p.m. of silica	0·0006*	0·0012†	0·00095	0·0009	0·0011
Degrees of freedom	50	62	9	10	10
Between-batch standard deviation, p.p.m. of silica	—	0·00135	0·0005‡	0·00035‡	0·0012
Degrees of freedom	—	50	9	9	9

* This figure is for reagent blank solution only and not for the difference: sample *minus* blank.

† One extremely low result excluded from the figures.

‡ Not statistically significant that a between-batch error exists.

Table III shows that both the within-batch and between-batch standard deviations were not significantly affected by the additional impurities present in the synthetic soft water samples. Detailed analysis of the results for the sodium silicate solutions showed that the standard deviations remained fairly constant over 2½ years. The between-batch coefficient of variation was about 0·25 per cent. and was possibly largely caused by variations in the temperature of the final solution; a ±2° C change in this temperature causes a ±0·3 per cent. change in optical density.[2]

Precision of the entire procedure—The entire procedure was repeatedly carried out on (*a*) sodium silicate solutions and (*b*) dilute clay suspensions (prepared from suspensions Nos. 1 and 2). For each solution or suspension, three portions were evaporated and fused in a batch (fusion batch) and the neutralised solutions from three fusion batches were treated together in a colorimetric batch. The optical densities of samples were corrected for reagent blank values and converted to p.p.m. of silica in the final solution; no corrections were made for the mean fusion blank value. The analyses were carried out during a period of 8 weeks, and statistical analysis showed no evidence for between-fusion batch and between-crucible effects except for the sodium silicate solution containing 0·211 p.p.m. of silica. The over-all standard deviations (those of one determination carried out in one colorimetric batch) were therefore calculated, and are shown in Table IV.

The standard deviations for clay suspensions 2A and 2B are considerably greater than those for the other solutions. This was possibly caused by non-uniform samples; the turbidity of suspension No. 2, from which the dilute solutions were prepared, supports this view, as

TABLE IV

PRECISION OF ENTIRE PROCEDURE

All concentrations are in the final solution

Type of sample	Volume of sample, ml	Silicon found, p.p.m. of silica	Degrees of freedom	Standard deviation, p.p.m. of silica	Coefficient of variation, %
Fusion blanks	—	0·019	38	0·0026	14
Triple-distilled water	20	0·023	24	0·0028	12
Clay suspension No. 2D† ..	20	0·062	8	0·0018	2·9
Sodium silicate solution ..	20	0·211	8	0·0015	0·7
Clay suspension No. 1A* ..	20	0·235	18	0·0032	1·4
Clay suspension No. 2C† ..	20	0·226	7	0·0034	1·5
Sodium silicate solution ..	2	0·403	8	0·0021	0·5
Clay suspension No. 2A† ..	2	0·426	7	0·0062	1·5
Sodium silicate solution ..	10	0·494	8	0·0035	0·7
Clay suspension No. 2B† ..	10	0·533	8	0·0056	1·1
Sodium silicate solution ..	2	0·571	7	0·0033	0·6

* Prepared by dilution of clay suspension No. 1.

† Prepared by dilution of clay suspension No. 2.

it indicates the presence of relatively large clay particles. These results were therefore rejected as not being representative of the precision attainable by the method, although they give information about the homogeneity of the samples.

The remaining results show that the standard deviation was relatively independent of silicon concentration in the final solution and of sample size.

BIAS ARISING IN EVAPORATION AND FUSION STAGES

The fusion procedure must convert all forms of silicon that might be present in power-station waters to sodium silicate or monomeric silicic acid. A direct test of this is difficult to devise, as these forms are not known; some possibilities are quartz, clays, magnesium silicates and polymeric silicic acid. A further difficulty is that of preparing solutions of known concentration of the substances tested. However, three kinds of tests for bias were made and are described below.

Fusion of standard substances in powder form—The weighed dried powders were fused with 0·5 or 1·0 g of sodium carbonate, the melt was partly dissolved in water and added to excess of acid as described under "Method," and then diluted to 1 litre in a calibrated flask. Suitable portions were then analysed by the colorimetric procedure. The standard silicon solution used for calibration purposes was analysed gravimetrically (two dehydrations with perchloric acid), and the determined concentration was used in the calculations; the results are shown in Table V. Although clay and felspar are the only two of the substances likely to be present in water, analysis of the other materials gives some idea of how the fusion copes with various siliceous substances.

Part of the bias shown in Table V may be due to errors in the gravimetric analysis of the standard silicon solution, in which the final weight of silica was only 0·1 g. If the Spectrosil rod is regarded as a standard—its impurity content is less than 1 p.p.m.—the maximum bias would be reduced to −2·5 per cent. The remaining bias is relatively small, and the cause has not been traced further; the effect of dissolving the melt in acid may be relevant to this problem (see "Experimental," p. 446).

Fusion of sodium silicate solutions—Fifteen 5-ml portions of standard silicon solutions containing 9·66 p.p.m. of silica were evaporated and fused as described under "Method." They were then treated by the colorimetric procedure together with further 5-ml portions of the same standard silicon solution. After correction for reagent blank values and the mean fusion blank value, the fused samples gave results 0·2 ± 1·0 per cent. lower than for those treated by the colorimetric procedure only.

Fusion of clay solutions—If a series of solutions of decreasing concentration is prepared by progressive dilution of one master solution, the relative concentrations of these solutions

Table V

Total silicon content of standard samples

Sample	Weight of sample, g	Weight of sodium carbonate, g	Silicon, as silica— certified, %	found, %		Difference, as percentage of silica content*
Spectrosil rod (synthetic fused silica)	0·01000	0·5	100·0	98·8		−1·2
American Bureau of Standards, Standard Samples—						
No. 98, Plastic clay	0·01000	0·5	59·11	57·4	57·3	−3·0
	0·1000	0·5		57·6		
	0·1000	1·0		57·2		
	0·1000	1·0		57·5		
	0·1000	1·0		57·0		
No. 99, Soda felspar	0·01000	0·5	68·66	67·7	67·15	−2·2
	0·1000	1·0		66·6		
No. 102, Silica brick	0·01000	0·5	93·94	92·0	92·4	−1·6
	0·1000	1·0		92·8		
No. 91, Opal glass	0·1000	1·0	67·53	65·5		−3·0

* For each determination, the 95 per cent. probability limits are estimated to be about ±0·6 per cent. of the silicon content; the differences from the certified values are, therefore, statistically significant.

will be known. Comparison of these known relative concentrations with those experimentally determined should reveal any bias that is not directly proportional to concentration. Such a series of results was obtained during the reproducibility tests carried out on dilute clay suspensions (see Table V). Table VI shows the relevant figures.

Table VI

Measured concentration of silicon in clay suspensions

Suspension No.	Volume of sample, ml	Total silicon in sample— Expected, p.p.m. of silica	Found,* p.p.m. of silica	Ratio of silicon found to silicon expected,* %
2	—	Used as standard	1790 ± 9	100 ± 0·5
2A	2	20·0	20·4 ± 0·2	102 ± 1
2B	10	4·99	5·14 ± 0·04	102·5 ± 0·8
2C	20	1·00	1·01 ± 0·01	101 ± 1
2D	20	0·20	0·20 ± 0·0075	100 ± 4

* Error limits are for ±ts at the 95 per cent. confidence level.

The results for suspensions 2A, 2B, 2C and 2D are consistent with one another, and, therefore, show no significant evidence of bias not proportional to concentration. However, they are not all consistent with the result for suspension No. 2. This could have been caused by bias, but, as it is not evident with the remaining suspensions, it is thought more likely to arise from the non-uniform nature of clay suspension No. 2, which could have caused an error when the concentrated solution was diluted. The results, therefore, indicate that, if bias is assumed to be constant, it is probably less than about 0·01 p.p.m. of silica in the sample.

Discussion of the work

With 20-ml samples, the criterion of detection and the limit of detection, which may be related to the standard deviation of the fusion blank value,[4] were about 0·02 and 0·04 p.p.m. of silica, respectively (95 per cent. confidence level; duplicate determinations). This is higher than ideally desirable, and it is useful to consider ways of improving the limit of detection by a factor of, say, 10. Examination of the precision figures given above shows that the largest contribution to the random errors at low concentrations was made by the fusion procedure; if its contribution had been zero, the criterion of detection would have been about 0·004 p.p.m. of silica (for 20-ml samples). Silicon in the sodium carbonate and contamination are thought to be the major sources of these errors, but as neither seems easy to reduce much further with a procedure similar to the proposed one, no more work was done on this aspect of the method.

A more valuable approach would be to increase the sample size. The errors arising in the fusion stage would be unaffected, and thus errors in terms of the concentration in the sample would be reduced. We have not used samples larger than 20 ml, as the time required for their evaporation would then become rather long. However, greater volumes may always be used when necessary; increased contamination during the longer evaporation time may then place a limit on the expected improvement in precision. There is clearly a need here for a means of rapidly evaporating a large volume of water on a small area of platinum, which is then subsequently fused with sodium carbonate.

The method is not suitable for use in the presence of more than about 0·05 to 0·1 p.p.m. of phosphate in the final solution.[2] To determine total silicon in the presence of phosphate, the fusion procedure could be combined with a reduced β-molybdosilicic acid absorptiometric finish.[2] The precision would then probably be worse, and special calibration samples would have to be taken through the entire procedure.

The method is fairly convenient to use, but requires about three times as many manipulations as "reactive" silicon methods. Four 20-ml samples can be analysed in duplicate per day when 3 platinum crucibles are used.

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2. ——, ——, *Ibid.*, 1963, **88**, 88.
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NOTE—References 1 and 2 are to Parts II and I of this series, respectively.

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The Analysis of Edible Oils Contaminated with Synthetic Ester Lubricants

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The analysis of edible oils contaminated or adulterated with synthetic ester oils used as lubricants (*e.g.*, for aviation gas-turbine engines) presents the analyst with novel problems. Unlike mineral lubricating oils, the presence of which can readily be detected, synthetic oils have many of the properties of edible oils, such as high saponification values.

Saponification and iodine values can be used as an early indication of contamination, and simple chemical techniques can be used to separate the contaminated oil into recognisable fractions. Properties are described of the saponification products of edible oils, synthetic ester lubricants and additives, such as tritolyl phosphate, as well as those of various unsaponifiable additives, such as phenothiazine and chlorinated biphenyl.

Thin-layer silica-gel chromatography is used to distinguish between synthetic ester oils, glycerides and mineral oils. Paper chromatography is used for detecting phenols (in the saponification products) and antioxidants containing nitrogen. Some applications of ultraviolet and infrared absorption spectrometry, mass spectrometry and gas - liquid chromatography are considered.

THERE have been occasions, fortunately rare, when edible oils have become contaminated, accidentally or deliberately, with lubricating oils. Analytical investigation can be troublesome because of the wide range of possible contaminants and the lack of any systematic method of isolating and identifying them.

Although most lubricating oils consist mainly of petroleum mineral oil, which can be readily detected as a contaminant of fatty oils,[1,2] some, such as aviation gas-turbine lubricants,[3,4,5] consist mainly of synthetic esters whose properties and reactions are similar to those of edible fatty oils. Moreover, most modern lubricating oils, whether of petroleum or synthetic origin, contain one or more of a wide variety of additives in sufficient quantity to complicate the analysis.

This paper presents a scheme of analysis utilising the essential chemical differences between fatty and synthetic ester oils for separating a mixture of such oils into identifiable fractions, and at the same time isolating and identifying additives likely to be present. The scheme not only gives an early qualitative indication of contamination, but permits its quantitative determination.

CHEMICAL CHARACTERISTICS OF VARIOUS EDIBLE OILS

The two most immediately useful parameters for assessing the purity of edible oils are saponification value[6] and iodine value.[7] Some typical values are listed in Table I.

TABLE I

SAPONIFICATION AND IODINE VALUES OF SOME EDIBLE OILS*

Oil	Saponification value, mg of potassium hydroxide per g	Iodine value, g of iodine per 100 g of oil
Palm (kernel)	242 to 252	14 to 19
Coconut	255†	7·0 to 9·5
Maize	188 to 193	103 to 125
Olive	188†	79 to 88
Groundnut	188†	82 to 99
Sesame	188 to 193	105 to 118
Soya bean	190†	129 to 143
Cottonseed	192†	103 to 113

* Values taken from B.S. 628–32 and 650–56 : 1950.
† Not less than.

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In general, whereas edible glycerides are esters of monocarboxylic acids (generally C_{12} to C_{22}) and glycerol, synthetic ester oils[3,4,5] are formed from—

(*i*) monohydric alcohols and dicarboxylic acids;

(*ii*) polyhydric alcohols (NOT glycerol) and monocarboxylic acids (generally C_4 to C_9).

Synthetic ester oils have saponification values greater than 250 and iodine values of zero. If a synthetic ester oil contaminates an edible oil, the saponification value of the latter will increase and its iodine value will decrease.* The change of saponification and iodine values with degree of contamination is considered later (p. 459).

CHEMICAL CHARACTERISTICS OF SYNTHETIC ESTER OILS

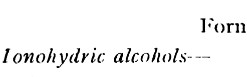
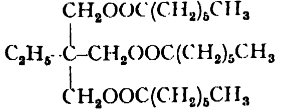
The major components of most synthetic ester oils are compounds of the types recorded in Table II. Some typical synthetic aviation turbine oil compositions are shown in Table III.

DISCUSSION OF TABLE II—

Table II records molecular weights, saponification values and saponification products of various synthetic ester oils. The saponification values are generally higher than those for glycerides. The molecular weights are much lower than those of the glycerides (about 884 for glycerin trioleate).

TABLE II

ESTERS

Formula	Molecular weight	Mols of KOH per mol	Saponification value	Hydrolysis products
Monohydric alcohols—				
Dioctyl sebacate $C_8H_{17}OOC-(CH_2)_8-COOC_8H_{17}$	426	2	263	2-Ethylhexanol Sebacic acid
Dinonyl sebacate $C_9H_{19}OOC-(CH_2)_8-COOC_9H_{19}$	454	2	247	3,5,5-Trimethylhexanol Sebacic acid
Dioctyl azelate $C_8H_{17}OOC-(CH_2)_7-COOC_8H_{17}$	412	2	272	2-Ethylhexanol or 2,2,4-Trimethylpentanol Azelaic acid
Dinonyl azelate $C_9H_{19}OOC-(CH_2)_7-COOC_9H_{19}$	440	2	255	3,5,5-Trimethylhexanol Azelaic acid
Dioctyl adipate $C_8H_{17}OOC-(CH_2)_4-COOC_8H_{17}$	370	2	303	2-Ethylhexanol Adipic acid
Dinonyl adipate $C_9H_{19}OOC-(CH_2)_4-COOC_9H_{19}$	398	2	282	3,5,5-Trimethylhexanol Adipic acid
Trihydric alcohols—				
1,1,1-Trimethylolpropane trivalerate  $\begin{array}{l} CH_2OOCC_4H_9 \\ \vert \\ C_2H_5-C-CH_2OOCC_4H_9 \\ \vert \\ CH_2OOCC_4H_9 \end{array}$	386	3	435	1,1,1-Trimethylolpropane Valeric acid
1,1,1-Trimethylolpropane tri-n-heptanoate  $\begin{array}{l} CH_2OOC(CH_2)_5CH_3 \\ \vert \\ C_2H_5-C-CH_2OOC(CH_2)_5CH_3 \\ \vert \\ CH_2OOC(CH_2)_5CH_3 \end{array}$	470	3	357	1,1,1-Trimethylolpropane n-Heptanoic acid

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* However, some synthetic ester oils, *e.g.*, dioctyl sebacate, have iodine values of zero and saponification values close to those of edible oils, such as coconut oil, that contain high proportions of the glycerides of lauric and myristic acids.

TABLE II: ESTERS—*continued*

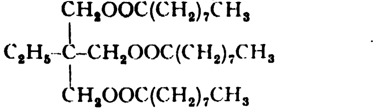
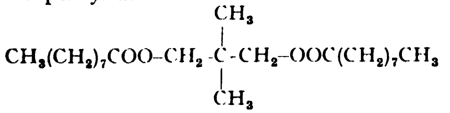
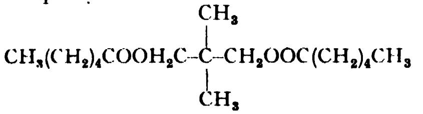
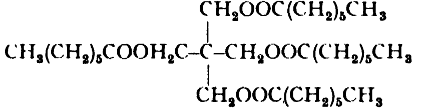
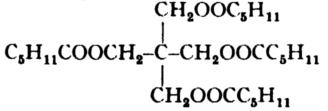
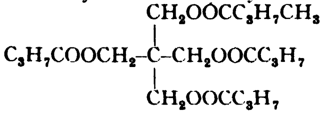
Formula	Molecular weight	Mols of KOH per mol	Saponification value	Hydrolysis products
1,1,1-Trimethylolpropane tri-n-nonanoate  $C_2H_5{-}\overset{\displaystyle CH_2OOC(CH_2)_7CH_3}{\underset{\displaystyle CH_2OOC(CH_2)_7CH_3}{C}}{-}CH_2OOC(CH_2)_7CH_3$	554	3	304	1,1,1-Trimethylolpropane n-Nonanoic acid
Neopentyl di-n-nonanoate  $CH_3(CH_2)_7COO{-}CH_2{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{C}}{-}CH_2{-}OOC(CH_2)_7CH_3$	384	2	292	Neopentyl glycol n-Nonanoic acid
Neopentyl di-n-hexanoate  $CH_3(CH_2)_4COOH_2C{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{C}}{-}CH_2OOC(CH_2)_4CH_3$	328	2	342	Neopentyl glycol n-Hexanoic acid
Polyhydric alcohols—				
Pentaerythritol tetra-n-heptanoate  $CH_3(CH_2)_5COOH_2C{-}\overset{\displaystyle CH_2OOC(CH_2)_5CH_3}{\underset{\displaystyle CH_2OOC(CH_2)_5CH_3}{C}}{-}CH_2OOC(CH_2)_5CH_3$	584	4	384	Pentaerythritol n-Heptanoic acid
Pentaerythritol tetra-n-hexanoate  $C_5H_{11}COOCH_2{-}\overset{\displaystyle CH_2OOC_5H_{11}}{\underset{\displaystyle CH_2OOCC_5H_{11}}{C}}{-}CH_2OOCC_5H_{11}$	528	4	424	Pentaerythritol n-Hexanoic acid
Pentaerythritol tetrabutyrate  $C_3H_7COOCH_2{-}\overset{\displaystyle CH_2OOCC_3H_7CH_3}{\underset{\displaystyle CH_2OOCC_3H_7}{C}}{-}CH_2OOCC_3H_7$	416	4	538	Pentaerythritol n-Butyric acid

TABLE III

TYPICAL SYNTHETIC ESTER OIL FORMULATIONS AND COMPONENTS

1.	Dioctyl sebacate	major constituent
	Phenothiazine Tritolyl phosphate	minor constituent
2.	Dinonyl sebacate	major constituent
	Thick ester based on polyoxyethylene glycol 200 M, sebacic acid and 2-ethylhexanol	minor constituent
	Phenothiazine	minor constituent
3.	Dioctyl sebacate	major constituent
	Thick ester based on 2-ethyl-2-methylpropane-1,3-diol, sebacic acid and 2-ethylhexanol	minor constituent
	Phenothiazine	minor constituent

4. (*a*) Thick esters
$C_8H_{17}OOC(CH_2)_8CO[(P.E.G.^*)OOC(CH_2)_8CO]_nOC_8H_{17}$

(*b*) $C_8H_{17}OOC(CH_2)_8CO[OC_7H_{14}OOC(CH_2)_8CO]_nOC_8H_{17}$

In both (*a*) and (*b*) the values of *n* can range from 1 to 6.

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* P.E.G. = polyoxyethylene glycol.

PRELIMINARY MEASUREMENT OF CONTAMINATION

Indications of contamination can often be obtained during the early stages of an analysis. Many synthetic ester oils after exposure to air develop characteristic colours; for instance, an oil containing phenothiazine can become deep red. The presence of substituted naphthylamines confers a bloom to an oil. Synthetic esters occasionally contain traces of free alcohols (*e.g.*, 2-ethylhexanol) and these impart characteristic sweet odours. Further, if after the determination of saponification value the saponification liquor has an ethereal odour (other than that of ethanol), the presence of octanols and nonanols is indicated.

The determination of such quantities as the saponification value, iodine value and phosphorus content of a suspect edible oil frequently allows the degree of contamination to be calculated, particularly when this is high. It is desirable that the saponification value and iodine value of an unadulterated specimen of the oil should be known. The type of calculation is shown below.

(*a*) *Effect of dioctyl sebacate on the saponification value of olive oil*—Contamination of olive oil by dioctyl sebacate (10 per cent. by weight) raises the saponification value from 192 (for example) to 199. If the saponification values for samples of olive oil received from the same area were normally 192, then this difference of 7 units would suggest contamination or admixture with another glyceride, *e.g.*, palm kernel oil.

(*b*) *Phosphorus content of olive oil contaminated with a synthetic ester oil containing tritolyl phosphate*—If a contaminating ester oil contains dioctyl sebacate and tritolyl phosphate (5 per cent. by weight), determination of the phosphorus content would give a figure of 0·043 per cent. by weight for 10 per cent. by weight contamination.

NOTE—It is unlikely that the analyst will encounter phospholipids in refined edible oils, hence the presence of phosphorus is usually an indication of contamination. However, some unrefined oils, *e.g.*, unrefined soya bean oil, contain phosphatides that on saponification yield glycerol, fatty acids, phosphoric acid and choline.

Most other synthetic ester oils will alter drastically the saponification values of most edible oils. However, the saponification values of an edible oil and an ester oil can be similar (*e.g.*, palm oil 242 to 252 and a dioctyl sebacate - tritolyl phosphate oil 260), and, in this instance, the saponification value is not a criterion of purity. Further, palm oil has a low iodine value and contamination with, for example, dioctyl sebacate would not alter it greatly. As before, a high phosphorus content would render such an oil suspect, but otherwise it is necessary to carry out more extensive analysis to detect contamination.

The presence in edible oils of contaminant additives such as tritolyl phosphate, diphenylamine, phenothiazine, silicon compounds and titanium compounds introduces alien elements. Given sufficiently sensitive methods, these elements can be quantitatively determined at low concentrations, and hence provide proof of contamination.

SCHEME OF ANALYSIS

A scheme for analysing mixtures of glycerides with synthetic esters is presented (see Table IV); it takes advantage of the ease of saponification of carboxylic esters. It permits both saponified and unsaponified materials to be separated into groups of compounds having common functional groups. Such compounds can frequently be isolated in a high degree of purity, making it possible to idenfify them by quantitative elemental microanalysis, by derivatives and by characterisation reactions. The nature of the original mixed esters can be ascertained from the composition of the saponification products.

The scheme of analysis is designed to meet most eventualities, and can often be simplfied on the evidence provided by the preliminary analyses. If the aim of the analyst is to give a rapid qualitative assessment of contamination, the determinations listed below will generally serve his purpose—

(*a*) Saponification value.
(*b*) Iodine value.
(*c*) Qualitative analysis of component acids.
(*d*) Qualitative analysis of hydroxy compounds.

The isolation of such compounds as amines, phenols and chlorohydrocarbons serves as further confirmation of adulteration. Although these compounds may be difficult to characterise fully, this in no way impedes the ester characterisation.

When members of homologous series of organic compounds, such as methyl esters of normal monocarboxylic acids, occur together, particularly when their boiling-points are close, separations are often extremely tedious, involving fractional crystallisation, vacuum distillation, etc. In these instances the more recent techniques, particularly gas - liquid chromatography, have come into prominence.[8] These methods are considered later (p. 465).

ANALYSIS OF FRACTIONS ISOLATED ACCORDING TO TABLE IV

These will usually be mixtures of octanols and nonanols. To date few other aliphatic alcohols appear to have been used in synthetic ester lubricants; sometimes a complex mixture of alcohols will be isolated, usually C_8 to C_{14}. The boiling-points of octanols and nonanols are close together, but the compounds can be separated by distillation. Their purity can be assessed from—

(*i*) Boiling-point determinations.

(*ii*) Active hydrogen or hydroxyl content (by acetylation).[9] Some properties of these alcohols are shown in Table V.

TABLE V

MONOHYDRIC ALCOHOL COMPONENTS OF SYNTHETIC ESTER LUBRICANTS

Alcohol	Molecular weight	Boiling-point, °C	Hydroxyl content, % by weight
2-Ethylhexanol	130	184	13·1
3,5,5-Trimethylhexanol	144	194	11·8
2,2,4-Trimethylpentanol	130	165	13·1

FRACTIONS 2 AND 10—

These fractions will consist of dicarboxylic acids. Determinations of equivalent-weights and melting-points can be used to identify pure acids, and will indicate the presence of mixtures. Methylation[10,11] of the acids with diazomethane, boron trifluoride - methanol or methanol - sulphuric acid will yield methyl esters, which can be separated by fractional distillation. Three dicarboxylic acids are commonly encountered in this type of analysis; they are listed in Table VI with some of their properties.

TABLE VI

PROPERTIES OF SOME DICARBOXYLIC ACIDS

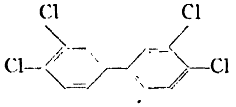
Acid	Molecular weight	Melting-point, °C	Boiling-point of diethyl ester, °C	Melting-point of *p*-nitrobenzyl ester, °C
Adipic	146	152	241	106
Azelaic	188	106	291	44
Sebacic	202	134	308	73

FRACTION 5—

This fraction will consist almost entirely of antioxidant materials containing nitrogen. These can be characterised by various colour reactions, melting-points, derivatives and nitrogen contents. Table VII contains details of the materials likely to be encountered in this fraction. They can also be analysed by paper chromatography.[12] Small portions of the nitrogen compounds (or the suspect oil) dissolved in acetone are spotted on a Whatman No. 1 paper impregnated with dipropylene glycol. The chromatogram is developed with cyclohexane saturated with dipropylene glycol. The individual compounds are located by spraying the paper with a solution of *p*-nitrophenyldiazonium fluoroborate in acetone. The resulting azo dyes have characteristic colours, which, together with R_F values, permit the amines to be identified.

FRACTION 6—

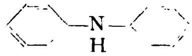
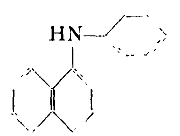
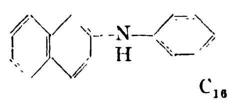
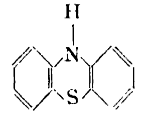
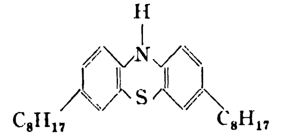
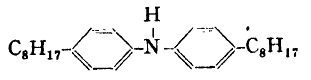
This fraction consists mainly of aromatic chloro-compounds. (Chlorinated polyphenyls and hydrocarbon oils rarely occur together.) The presence of chloro-compounds is readily indicated by means of Beilstein's copper wire test. Elemental analysis will confirm an aromatic nucleus. A typical material likely to appear in this fraction would be—



The elemental analysis is C, 49·3 per cent.; H, 2·1 per cent.; Cl, 48·6 per cent. The low hydrogen content indicates an aromatic nucleus. Any mineral oil present in a contaminated edible oil could appear in this fraction.

TABLE VII

PROPERTIES OF SOME ANTIOXIDANTS CONTAINING NITROGEN

Compound	Formula	Molecular weight	Nitrogen, % by weight	Sulphur, % by weight	Melting-point, °C	Melting-point of derivative, °C	Other details*
Diphenylamine	 $C_{12}H_{11}N$	169	8·28	—	54	N-acetyl 198	R_F 0·55
N-phenyl-1-naphthylamine	 $C_{16}H_{13}N$	219	6·39	—	62	N-acetyl 115	R_F 0·57
N-phenyl-2-naphthylamine	 $C_{16}H_{13}N$	219	6·39	—	108	N-acetyl 93	R_F 0·43
Phenothiazine	 $C_{12}H_9NS$	199	7·03	16·1	185	—	Ratio sulphur to nitrogen = 2·29 R_F 0·19
3,7-Dioctylphenothiazine	 $C_{28}H_{41}NS$	423	3·31	7·56	168	—	Intense carmine colour with conc. H_2SO_4 R_F 0·82
Di-(*p*-octylphenyl)-amine	 $C_{28}H_{43}N$	393	3·56	—	104	—	R_F 0·86

* R_F values obtained by using Delves system.[12]

FRACTION 7—

This fraction will contain compounds such as polypropylene oxide, polyethylene oxide or copolymers of ethylene oxide and propylene oxide—

$$HO(C_3H_6O)_n(C_2H_4O)_mH$$

where *n* and *m* may be any integral number, but values between 4 and 20 are to be expected.

Polyethylene oxide [$HO(C_2H_4O)_nH$] has a carbon to oxygen ratio of between 1 and 2, whereas polypropylene oxide has a carbon to oxygen ratio of between 1·5 and 3, the exact values being dependent on molecular weight.

A copolymer of both oxides will have a carbon to oxygen ratio falling within the range 1 to 3, but its molecular weight will be too low for the corresponding value for polypropylene oxide and too high for the corresponding value for polyethylene oxide. Thus a knowledge of the molecular weight and carbon to oxygen ratio of a polymer can assist in its characterisation.

On oxidation with a potassium dichromate - sulphuric acid mixture, polypropylene oxide and its copolymer with ethylene oxide give a copious evolution of acetaldehyde. A pure ethylene oxide polymer undergoes rapid oxidation, but does not form acetaldehyde. Polyethylene oxides are usually readily soluble in water, whereas polypropylene oxides are not.

FRACTION 8(*a*)—

This fraction contains phenolic material, which will only be present if aryl phosphates or alkylaryl phosphates occurred in the untreated oil. It will have a characteristic carbolic odour, and give a green or purple colour with aqueous ferric chloride solution. In all probability it will be a complex mixture of phenol, cresols, xylenols and higher phenols. Resolution of such a mixture by conventional means is almost impossible on the small amounts of material isolated. However, it can be analysed readily by paper chromatography.[13] The phenols are coupled in alkaline media with diazotised *p*-nitroaniline; the solution is then acidified with dilute mineral acid and the dyes are extracted with ether. A small portion of the azo-dye mixture is spotted on a Whatman No. 1 paper impregnated with 20 per cent. v/v of formamide in acetone and developed with cyclohexane - benzene solution (70 + 30 by volume) containing 3 per cent. v/v of dipropylene glycol. The azo-dyes are characterised by R_F values and the colour reactions of the spots with ammonia or aqueous sodium carbonate.

Gas - liquid chromatography can also be used to resolve small amounts of complex mixtures of phenols.[14]

FRACTION 8(*b*)—

This fraction contains any low molecular weight carboxylic acids present. The acids most likely to be encountered are mentioned below.

They can be divided into two groups—

(*i*) water-soluble acids	n-butyric acid isovaleric acid n-valeric acid n-hexanoic acid
(*ii*) water-insoluble acids	n-heptanoic acid n-octanoic acid n-nonanoic acid

The two groups can be further separated into their pure components by distillation of the methyl esters.

The acids isolated will usually be a mixture, *e.g.*, hexanoic, heptanoic and nonanoic acids, so that equivalent-weight determinations will give average values. Gas - liquid chromatographic separations of the methyl esters[15] is the most rapid method of quantitative analysis for each component.

FRACTIONS 3 AND 9—

These fractions contain polyhydroxy material. If the oil being tested contains any fat at all, then glycerol will be present and can be oxidised quantitatively by potassium periodate. This fraction also contains potassium salts and excess of mineral acid, which must be neutralised before proceeding further. The aqueous solution of salts and hydroxy compounds is evaporated to dryness, under vacuum, on a steam-bath. The resulting salt cake is dried by azeotropic distillation with anhydrous isopropyl alcohol, and the hydroxy compounds are then dissolved with the same solvent. (Alternatively, the ionic material can be removed by percolating the saline - acid solution through ion-exchange resins, *e.g.*, Amberlite 400 (OH^-) and Amberlite 120 (H^+).) The isopropyl alcohol is distilled off, and any hydroxy compounds remaining can be characterised. Any glycerol present can be destroyed by periodate oxidation (prolonged treatment can also degrade pentaerythritol). Table VIII shows the properties of the most common hydroxy compounds that will be encountered in this fraction.

FRACTION 11—

This fraction contains high molecular weight monocarboxylic acids derived from glycerides. Some properties of these acids are shown in Table IX.

TABLE VIII

PROPERTIES OF SOME POLYHYDROXY COMPOUNDS

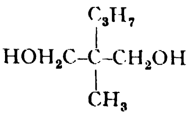
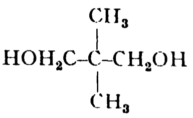
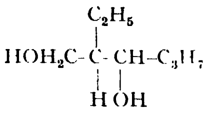
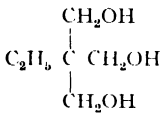
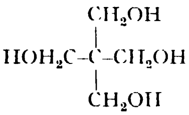
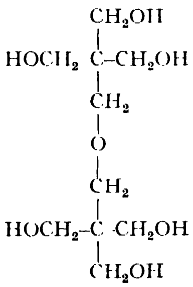
Compound	Formula	Molecular weight	Melting-point, °C	Boiling-point, °C	OH, % by weight
2-Isopropyl-2-methyl-propane-1,3-diol	 $HOH_2C-C(C_3H_7)(CH_3)-CH_2OH$	132	58	—	25·8
2,2-Dimethylpropane-1,3-diol	 $HOH_2C-C(CH_3)_2-CH_2OH$	104	123	—	32·7
2-Ethylhexane-1,3-diol	 $HOH_2C-C(C_2H_5)(H)-CH(OH)-C_3H_7$	146	Liquid	134 (5 mm)	23·3
1,1,1-Trimethylolpropane	 $C_2H_5-C(CH_2OH)_3$	134	59	—	38·1
Pentaerythritol	 $HOH_2C-C(CH_2OH)_2-CH_2OH$	136	261 to 262	—	50·0
Dipentaerythritol	 $HOCH_2-C(CH_2OH)_2-CH_2-O-CH_2-C(CH_2OH)_2-CH_2OH$	254	222	—	40·1
P.E.G. 200	Mainly tetra-ethylene-glycol	194	Liquid	328	17·5

TABLE IX

PROPERTIES OF HIGH MOLECULAR WEIGHT FATTY ACIDS

Acid	Molecular weight	Boiling-points of methyl esters, °C	Other properties
Lauric	200	87 (1 mm)	*p*-bromophenacyl ester m.p. 76° C
Palmitic	256	111 (1 mm)	*p*-bromophenacyl ester m.p. 84° C
Stearic	270	130 (1 mm)	*p*-bromophenacyl ester m.p. 90° C
Oleic	268	160 to 162 (2 to 3 mm)	—
Linoleic	266	168 to 170 (1 mm)	Tetrabromo-derivative m.p. 180° C

The chemical separation and identification of these acids in mixtures is extremely tedious. The application of gas - liquid chromatography[8,15,16] and sometimes thin-layer chromatography[17] quickly gives both qualitative and quantitative identification of each component acid.

EXAMPLE OF AN ANALYSIS

Let us assume that an olive oil (saponification value 192) contains 50 per cent. by weight of a synthetic ester oil having the composition—

94 per cent. by weight of dioctyl sebacate
5 per cent. by weight of tritolyl phosphate } Saponification value about 260*
1 per cent. by weight of phenothiazine

The saponification value of the mixed oils will be 226, and the saponification liquor will have a strong odour of octanols. The iodine value (say 82) of the olive oil will be decreased to 41 by the synthetic ester. The phosphorus content of the oil will be 0·210 per cent. by weight. In all probability the untreated oil mixture will also have an odour of octanols and will be red.

The fractions listed below will be obtained when the oil is analysed—

Fraction 4—Octanol (2-ethylhexanol).

Fraction 5—Phenothiazine. (The silica gel column used to prepare this fraction will be pale green after elution with acetone as phenothiazine is present.)

Fraction 8(*a*)—Phenols.

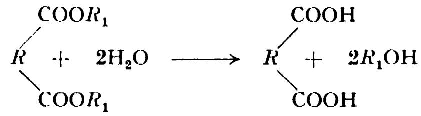
Fraction 10—Sebacic acid.

Fraction 11—C_{16}, C_{18} saturated and unsaturated fatty acids.

Fraction 9—Glycerol.

Fractions 4, 10 and 11 can be weighed and fraction 9 determined by periodate oxidation. The phenothiazine can be characterised by paper chromatography (on the original oil mixture) and determined via the sulphur content of the oil (0·5 per cent. by weight of phenothiazine ≡0·08 per cent. by weight of sulphur).

Tritolyl phosphate can be determined from the phosphorus content of the oil (1 per cent. by weight of tritolyl phosphate ≃0·084 per cent. of phosphorus), and the constituent phenols can be characterised (after saponification) by paper chromatography.[13] In balancing the weights of the fractions, allowance must be made for the water gained on saponification, *e.g.*—



$$R(COOR_1)_2 + 2H_2O \longrightarrow R(COOH)_2 + 2R_1OH$$

If this allowance is not made the mass balance should exceed 100 per cent. recovery (a low recovery is probably due to the volatility of fraction 4).

RECOVERY OF TRITOLYL PHOSPHATE—

If it is required to concentrate the tritolyl phosphate this can be achieved by percolating the suspect oil through silica gel. Tritolyl phosphate is strongly adsorbed, whereas most of the esters are not and can be eluted with benzene. Some glycerides however are eluted with tritolyl phosphate, *e.g.*, glycerides of erucic acid found in mustard oils. The tritolyl phosphate is desorbed with acetone, and about a five-fold concentration is achieved, which facilitates mass spectrometric analysis. A quantitative assessment of the molecular-weight distribution of aryl phosphates present can thereby be obtained (see Table X).

TABLE X

MASS SPECTROMETRIC ANALYSIS OF A COMMERCIAL TRITOLYL PHOSPHATE

Molecular weight	Distribution, %	Phosphorus compound (typical of molecular weights in column 1)	Phosphorus, %
340	0·1	Diphenyl tolyl phosphate	9·12
354	0·5	Phenyl ditolyl phosphate	8·75
368	41·5	Tritolyl phosphate	8·43
382	54·0	Ditolyl xylyl phosphate	8·12
396	2·9	Tolyl dixylyl phosphate	7·84
410	0·4	Trixylyl phosphate	7·57
424	0·3	Higher phosphate	7·33

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* Tritolyl phosphate will also be saponified.

MORE RECENT ASPECTS OF ANALYSIS

Although the scheme of analysis outlined permits the edible oil contaminants to be characterised within narrow limits and allows a quantitative determination of contamination to be made, it still poses analytical problems that are not readily solved. For example, the complex mixture of acids isolated as Fractions 10 and 11 can only be separated chemically by tedious means, and fractional distillation is slow. Further, such methods are usually only semi-quantitative. The quantitative analysis of such mixtures of acids has been radically simplified by the use of gas - liquid chromatography,[8,15,16] which will separate the methyl esters of mixtures of closely related acids, such as azelaic and sebacic acid, or stearic, oleic, linoleic and linolenic acids.

Gas - liquid chromatography can also be used to separate octanols and nonanols (*e.g.*, Fractions 1 and 4), and resolve a complex mixture such as cresylic acid[14] (Fraction 8(*a*)).

If the contaminant esters are dioctyl sebacate, dinonyl sebacate or dinonyl adipate they can be identified by gas - liquid chromatography without the need for hydrolysis. Phenothiazine, diphenylamine and possibly substituted diphenylamines can also be determined by this technique.

A valuable method of determining secondary aromatic amines is ultraviolet spectroscopic analysis. Minute concentrations of amine compound, *e.g.*, $<0{\cdot}01$ per cent. of phenothiazine, can be determined in the untreated oil. Although some unsaturated carboxylic acids also absorb in the ultraviolet region, they do not interfere.

Infrared spectroscopic analysis affords clear preliminary indications of the major functional groups present in the contaminated oil and in the various fractions, *e.g.*, the aromatic character of chlorinated biphenyls is readily shown.

Mass spectrometric analysis can be extremely useful in the examination of such components as nitrogen compounds and aryl phosphates.

A rapid assessment of the possibility of contamination can be obtained by the use of thin-layer silica-gel chromatography.[18] Edible oils and synthetic ester oils give characteristic chromatograms that can be readily distinguished, and hence the presence of the synthetic ester is detected. Further, when the chromatogram is sprayed with *p*-nitrophenyldiazonium fluoroborate, most antioxidants present can be revealed as brightly coloured zones.

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The Rapid Alkaline Reduction of Nitro-groups and the Kjeldahl Determination of Nitrogen

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Alkaline stannite rapidly reduces certain nitrophenols so that the nitrogen can be quantitatively determined by the Kjeldahl method. The method may permit easier analysis of moisture-sensitive compounds.

This appears to be the first recorded pre-treatment in alkaline solution, although many acidic methods have been described. These methods are reviewed briefly, with special reference to the determination of nitrogen in isomeric nitrophenols.

ALTHOUGH the Kjeldahl nitrogen determination was discovered in 1883 and now incorporates additional features to broaden its scope, there are still certain types of nitrogen compounds with the element in a more oxidised form, and a few heterocyclic structures, pyridine and acridine,[1] which do not yield all their nitrogen as ammonium sulphate in any of the Kjeldahl modifications.

Various pre-treatments are used for nitro-, nitroso-, azo-, oximino- and similarly bound nitrogen compounds. Most are acid reductions: with sulphur and sulphuric acid[2]; zinc and fuming sulphuric acid[3]; iron filings and sodium oxalate and sodium formate in sulphuric acid[4]; stannous chloride and hydrochloric acid in a sealed tube at 120° C[5,6]; steel filings, copper oxide and 18 N sulphuric acid[7]; zinc and hydrochloric acid or a non-oxidising acid[8,9,10]; alcoholic sodium dithionite ($Na_2S_2O_4$)[11,12]; activated copper and zinc couple[13]; glucose, on a semi-micro and micro scale[14,15]; but compare Takeda and Senda[16] and Abrahamczik[17]; chromous chloride, obtained as required from chromic chloride and zinc amalgam[18]; titanous chloride[19]; hypovanadous salts[20]; phosphonium iodide[21]; hydrogen and a nickel catalyst.[22] Acid reduction in the final stage has also been employed.[23]

The work of Margosches and co-workers has shown that *o*-hydroxynitro-compounds are degraded completely, whereas a *p*-oriented hydroxy-group does not destabilise the ring sufficiently for quantitative analysis; *m*-isomers are similar to the latter.[24] These workers also showed that addition of phenolsulphuric acid without heating, for an initial period, afforded quantitative analyses of nitrates.[25] Resorcinol or phloroglucinol were also shown to sensitise nitrates to degradation.[26] This is doubtless due to nitration of the strongly nucleophilic phenols. The use of salicylic acid,[23] *o*-mercaptobenzoic acid[27,28] or a mixture of equal amounts of 1-naphthol and pyrogallol[29]—see also Tirouflet[30]—has been found to ensure quantitative decomposition of aromatic nitro-compounds. Their successful use is to be connected with reversible de-nitration of the nitro-compound, with subsequent more rapid nitration of the phenols, which are then decomposed by the medium of hot sulphuric acid. The thiol group of the acid $C_6H_4(SH)CO_2H$ exerts a separate reducing action.

Some of these pre-treatments employed have also found use with osazones[31]; azo-compounds[11,19,32]; hydrazine and its uni- and bivalent substituted products.[7,33,34]

Other Kjeldahl pre-treatments employed are addition of formaldehyde to an azide degradation to prevent loss of gaseous nitrogen[35]; conversion of organic thiocyanates to cuprous thiocyanate, which is then more readily digested[36]; gradual oxidation with manganese dioxide in the Kjeldahl determination[37]; oxidation with potassium permanganate below 85° C, effective also with pyridine compounds, to ammonium and nitrate ions. The latter are then reduced with a Raney alloy[38]; alkaline peroxide also aids decomposition of pyridines[39]; addition of benzoic acid in the degradation of benzonitrile, benzophenoneoxime and azobenzene,[40] and addition of cuprous oxide.[41]

During the analysis of some organo-boron compounds obtained from boron trichloride and phenols and phenols with *o*- or *p*-nitro-groups or both, *e.g.*, $(NO_2.C_6H_4O)_xBCl_{3-x}$, where x was 1 or 3, the determination of boron was hindered on account of volatile nitrophenols distilling over with methyl borate.[42] *p*-Nitrophenol as well as the *o*-isomer and 2,4-dinitrophenol are sufficiently volatile in methanol to collect in the condensate and, because of the deep yellow colour of the phenolate ion, obscure the phenolphthalein end-point of a titration of boric acid and mannitol with sodium hydroxide.

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To prevent this, and to obtain the nitrogen in a form suitable for determination by the Kjeldahl method, the nitro-compound (0·2 to 0·4 g) was reduced with alkaline stannite. Approximately 1·5 times the stoicheiometric weight of stannous chloride hexahydrate necessary for complete reduction to amine was dissolved in the minimum of hydrochloric acid with warming. The concentrated acid was used, but more dilute acid might suffice. After the solution had been cooled, 30 per cent. sodium hydroxide solution was added carefully, with shaking and cooling in cold water (under a running tap). At first, crystalline sodium chloride was precipitated; almost at once, on further addition of alkali, a pale yellow curdy precipitate was formed, and no further alkali was added after the precipitate had dissolved. This solution was added immediately to the sample to be reduced in aqueous methanol containing a drop of alkali. Reduction was complete after the solution has been warmed gently for a few moments; occasionally, stronger heating to the boil with swirling may be required and the process will be complete when the deep yellow colour has been discharged. This was always a definite change. This solution was cooled and diluted with water before addition of concentrated sulphuric acid and catalyst for a Kjeldahl reaction, or with methanol for the determination of boron.

If, in the preparation of the alkaline stannite, a darkening and precipitation of fine black particles occurred, indicating disproportionation to metallic tin and stannate, it was found better to prepare a fresh sample. This disproportionating, which is accelerated by warming, may be avoided by slower addition of the alkali.

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The Quantitative Determination of Non-ionic Detergents of the Type Formed by the Condensation of Nonyl Phenol with Ethylene Oxide

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The method described involves the measurement of the optical density, at 490 mμ, of dilute solutions of dichlorofluorescein in acetic acid; small amounts of non-ionic detergent of the type formed when nonyl phenol is condensed with ethylene oxide appreciably reduce the optical density, and this effect can be used in determining the amount of detergent in an unknown solution. Anionic and other non-ionic detergents in similar amounts have a much smaller effect on the optical density. A calibration curve must first be prepared from known amounts of the detergent. The effect of the detergent on optical density is dependent on pH, and the sensitivity of the method can be altered by working at different pH values, with acetic acid - sodium acetate buffer solutions.

NON-IONIC detergents of the Lissapol N type are widely used in New Zealand for scouring raw wool and in the later stages of wool processing. Methods are available for the quantitative determination of these materials, usually by gravimetric analysis,[1,2] but they are not recommended for amounts of less than 10 mg. Other more sensitive absorptiometric methods are available in which the colour of the complex formed by the detergent with molybdophosphoric acid[3] or 2,4-dinitrophenylhydrazine[4] is measured to determine the amount of detergent in a mixture. Schonfeldt[5] also describes a method dependent on the addition product formed when the ethylene oxide chain of the detergent molecule reacts with ferrocyanic acid, the excess of acid being determined by titration with zinc sulphate. The method described in this paper, though sensitive to one type of non-ionic detergent only, has the advantages of simplicity and speed.

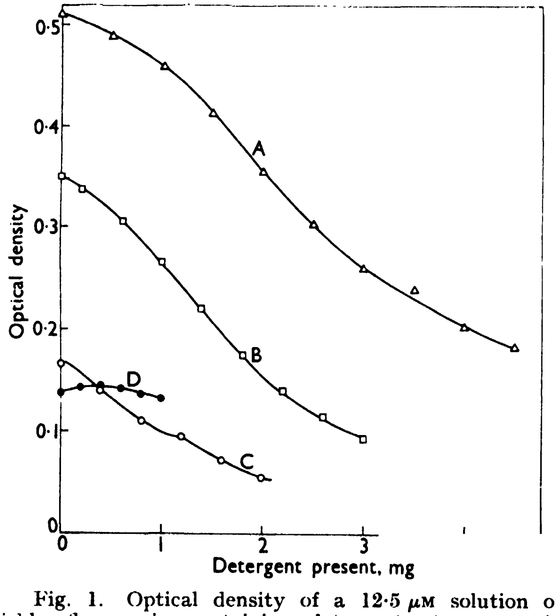

Fig. 1. Optical density of a 12·5 μM solution of dichlorofluorescein containing detergent at: curve A, pH 4·65; curve B, pH 4·0; curve C, pH 2·9; curve D, pH 2·6

Lovelock and Nash[6] reported that traces of some substances, among them some anionic detergents, appreciably reduced the optical density of a dilute solution of dichlorofluorescein in acetic acid. They attributed this to micelle formation and suggested that the reaction might be applied to the quantitative determination of non-ionic detergents. This was investigated in these laboratories and found to be a quick and accurate method of quantitatively determining detergents of the type formed when nonyl phenol is condensed with ethylene oxide.

EXPERIMENTAL

An approximately 0·005 M solution of dichlorofluorescein in glacial acetic acid was prepared, and 2·5 ml were diluted to 500 ml in a calibrated flask. For an investigation of the effect of pH, known amounts of acetic acid or sodium acetate were added to this solution before it was made up to the mark.

By pipette, 25 ml of the diluted dichlorofluorescein solution were transferred to a 50-ml calibrated flask containing a known amount of detergent, and the solution was diluted to the mark with distilled water. The optical density of the solution was measured in 1-cm Corex cells with a Beckman DU spectrophotometer at 490 mμ against distilled water. By using, initially, the non-ionic detergent Lissapol N450 curves of optical density against concentrations of detergent were prepared (see Fig. 1). The pH shown is that of the dichlorofluorescein - detergent solution whose optical density was being measured.

From an examination of the results obtained it was concluded that the measurement of the optical density at pH 4 would provide a satisfactory method for the quantitative determination of the detergent.

METHOD

The method can be used to determine detergent in the range 0·2 to 3·0 mg.

REAGENTS—

Dichlorofluorescein.
Acetic acid, glacial.
Sodium acetate, crystalline.

PROCEDURE—

Weigh out 100 mg of dichlorofluorescein, and dissolve in 30 ml of glacial acetic acid, warming if necessary. Cool, transfer to a 50-ml calibrated flask, and make up to the mark with glacial acetic acid. Transfer 2·5 ml of this solution by pipette to a 500-ml calibrated flask containing 1·532 g of sodium acetate dissolved in approximately 450 ml of distilled water, and make up to the mark. This solution is stable for at least 24 hours.

By pipette, transfer 25 ml of this diluted dichlorofluorescein solution to separate 50-ml calibrated flasks containing known amounts (0·2 to 3 mg) of detergent, dilute each solution to the mark with distilled water, and measure the optical densities at 490 mμ against distilled water. Prepare a calibration graph of optical density against amount of detergent present.

Take a suitable sample of a solution of unknown detergent concentration, add 25 ml of dichlorofluorescein solution, dilute to 50 ml with distilled water, and measure the optical density. By reference to the calibration graph, determine the amount of detergent in the sample solution.

A more sensitive variation of the proposed method is made possible (range 0·1 to 1·0 mg) by omitting the sodium acetate. However, the disadvantages are that the diluted dichlorofluorescein solution is not particularly stable and must be used within 2 hours, and that the spectrophotometer cell must be well rinsed with dilute alkali and distilled water after each optical-density measurement.

DISCUSSION OF RESULTS

An examination of Fig. 1 shows that, at pH 2·6, the curve obtained is of no value for the quantitative determination of detergent.

At pH 2·9 a large change in optical density was associated with a small change in detergent concentration. The curve shows a shoulder in the region of 1 mg of detergent, but it was found possible to use this curve for determining amounts of less than this. Recovery of detergent was reasonably good (see Table I), but, at this pH, contamination of the cell surface owing to adsorption of dichlorofluorescein occurred fairly rapidly, and it was necessary to clean the cell with dilute alkali after each determination. Also, the dilute solution of dichlorofluorescein deteriorated after a short length of time and had to be used within 2 hours of preparation.

TABLE I

RECOVERY OF LISSAPOL N450 AT pH 2·9

Lissapol N450 present, mg	0·30	0·50	0·70	0·90
Lissapol N450 found, mg	0·28	0·48	0·71	0·92
Recovery, %	93	96	101	102

The optical density at higher pH values was not so sensitive to the presence of detergent, but the usable range was greater. Also, dichlorofluorescein was not adsorbed on the cell walls to the same extent, and the diluted solution could be kept for at least 24 hours without deterioration. Thus measurement of optical density at pH 4 forms the basis of the proposed method. Table II shows the recovery obtained with three commercially available detergents of the same type.

TABLE II

RECOVERY OF DETERGENT AT pH 4

	Lissapol N450 (I.C.I.)		Renex 688 (Atlas)		Triton N100 (Rohm and Haas)	
Detergent present, mg ..	1·20	2·40	1·20	2·40	1·20	2·40
Detergent found, mg ..	1·24	2·35	1·22	2·37	1·21	2·38
Recovery, %	103	98	102	99	101	99

The scope of the method is unfortunately narrow, since it was found that only the non-ionic detergents formed by the condensation of nonyl phenol with ethylene oxide could be determined. Other non-ionic detergents, *e.g.*, condensation products of tridecyl alcohol with ethylene oxide and detergents of the alkylolamide type, had only a relatively small effect on the optical density of dichlorofluorescein solutions. A few anionic detergents were also examined, among them primary and secondary alkyl sulphates and salts of fatty acids. These too had only a small effect on the optical density, reducing it by approximately 5 per cent. of the reduction expected when the same amount of non-ionic detergent of the suitable type was present. Because of this small effect exerted by other detergents the method is not applicable to mixtures containing appreciable unknown amounts of other non-ionic or anionic detergents. Cationic detergents brought about a change in colour to rose pink, owing to salt formation, and would also interfere in any determination. The presence of some water-miscible solvents (those investigated were ethanol, isopropanol and acetone) in comparatively large amounts also reduced the optical density; 5 ml of each solvent were equivalent to approximately 0·7, 0·9 and 1·2 mg of the non-ionic detergent, respectively. However, such interference could, presumably, be easily avoided by evaporating the solvent from the sample of detergent before it was diluted.

The reduction in optical density when non-ionic detergents of a suitable type were present appeared to depend on the hydrophobic portion of the detergent molecule. This was indicated when it was found that substituting tridecyl alcohol for nonyl phenol greatly reduced the effect. A series of non-ionic detergents was examined of which the hydrophobic portion of the molecule in each instance was nonyl phenol, but the hydrophilic portion consisted of ethylene oxide chains of different lengths (Renex detergents obtainable from Atlas Powder Co.). When equivalent amounts of these detergents were used, the reduction in optical density was similar for each detergent.

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The Determination of Traces of Boron in Zirconium and Zirconium Alloys

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A general procedure is described for determining down to 0·2 p.p.m. of boron in zirconium and zirconium alloys. Separation of the boron is not necessary, as the boron - curcumin complex is formed directly in a portion of the metal sulphate solution.

An interference effect has been noted when zirconium alloys containing tin are analysed. The interference is caused by an insoluble compound of curcumin, which separates and has similar properties to those of the boron - curcumin complex. This source of interference is, however, readily overcome in the proposed procedure.

The proposed procedure has been used for determining boron in pure zirconium, zirconium - 0·5 per cent. of copper - 0·5 per cent. of molybdenum and zirconium - 1·5 per cent. of tin - 0·1 per cent. of iron - 0·1 per cent. of chromium - 0·05 per cent. of nickel alloys. Results were similar to those obtained by methods in which the boron is separated as methyl borate.

THE introduction of zirconium and zirconium alloys as structural materials in nuclear reactors has made necessary the determinations of extremely small concentrations of those impurities having high thermal neutron absorption cross-sections. One such impurity is boron, the concentration of which is normally limited to less than 1 p.p.m.

Most methods for determining small amounts of boron involve a separation step and then the spectrophotometric measurement of the complex formed with a sensitive reagent. Separation has been achieved by ion exchange,[1] solvent extraction[2,3] or, more usually, distillation as methyl borate by modifications of Gooch's method.[4] Procedures have been described for determining boron in zirconium and zirconium alloys,[5,6] and these involve the separation of boron by distillation as methyl borate.

After an investigation of the boron - curcumin reaction, we proposed more sensitive and reliable methods for determining boron with curcumin.[7] These procedures were applicable to determining boron in a solution of the salts of several metals, without the time-consuming and laborious separation as methyl borate. The procedures involved development of the boron colour at room temperature in an acetic acid - sulphuric acid medium, after which the solution could be diluted with alcohol for measurement of the colour. Alternatively, dilution with water resulted in precipitation of the boron complex (rosocyanin), which could be filtered off and dissolved in a small volume of alcohol for measurement, thus giving greater sensitivity. The maximum amount of aqueous sample solution that could be present at the colour development stage was 0·25 ml, and this placed the lower limit for determining boron in metals at about 1 p.p.m., which is seldom low enough for reactor materials.

Relatively simple modifications to these procedures have permitted the direct determination of boron in zirconium and zirconium alloys to be made down to the 0·2 p.p.m. level. During this work, apparently high results for boron were obtained from zirconium alloys containing tin; this effect was shown to be caused by the presence of a curcumin compound of unknown composition. The interference caused by the tin was easily overcome by virtue of the solubility of the tin - curcumin compound in hydrochloric acid, in which the boron - curcumin complex is insoluble.

A study of the formation of the compound formed in the presence of tin is currently being made.

METHOD

REAGENTS—

Curcumin - acetic acid reagent—Dissolve 0·125 g of curcumin in 100 ml of analytical-reagent grade glacial acetic acid with warming and stirring. The reagent is stable for several months. (Curcumin obtained from Hopkin and Williams Ltd. was used without further purification.)

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Sulphuric acid—Sulphuric acid, AsT, sp.gr. 1·84.

Methylated spirit—Industrial grade 70 O.P. to which has been added 0·1 per cent. v/v of acetic acid.

Water, boron-free—Prepare by passing distilled water through a column of mixed-bed ion-exchange resin.

All other reagents should be of analytical grade.

FILTER COLUMN FOR SEPARATING ROSOCYANIN—

The column used for separating the boron complex is that described previously.[7] Make a slurry of asbestos (grade A long-fibre Powminco—obtainable from Hopkin and Williams Ltd.) with water, allow to settle for a few minutes, and then decant and discard the fines. Place sufficient of the slurry in the column to produce a pad about 0·5 cm thick when gentle suction is applied.

PROCEDURE—

Dissolve 1·0 g of the sample by heating under reflux with 5·0 g of ammonium sulphate and 15 ml of sulphuric acid in a small all-silica apparatus. Allow the resulting solution to cool, dilute to 40 ml with sulphuric acid, mix well, and store in a polythene bottle.

Put 2·5 ml of the sample solution into a platinum dish, and stand the dish on a beaker containing cold water in such a way that the bottom of the dish touches the surface of the water.

Add 3·0 ml of curcumin - acetic acid reagent from a burette, mixing thoroughly during the addition with a small polythene rod. Remove the dish from the beaker, and set aside for 15 minutes at room temperature. Rapidly add 10 ml of diluted hydrochloric acid (3 + 1), mix well, and filter through a prepared column. Allow the column to drain (gentle suction may be used if necessary, but the rate of filtration should not exceed about one drop per second). Rinse the dish with a further 10 ml of diluted hydrochloric acid (3 + 1), pass it through the column, and then allow to drain. Rinse the dish with 20 ml of water, place the rinsings on the column, and then allow to drain.

Rinse the dish well with di-ethyl ether, place the rinsings on the column, and then continue washing the column with ether until the eluate is colourless (this indicates freedom from excess of curcumin). Wash the column with 10 ml of concentrated hydrochloric acid and then with water to remove all acid; drain the column well under gentle suction.

Place 5 ml of methylated spirit in the dish, swirl, and then run it through the column; collect the eluate in a 10-ml stoppered cylinder. Continue adding methylated spirit to the column, a few millilitres at a time, until 10 ml of eluate have been collected. Mix well, and measure the optical density at 555 mμ in a 4-cm cell. Correct the optical density for the reagent blank value determined by carrying out the procedure with all the reagents, but omitting the sample. By using the reagents stated the value of the blank optical density should normally be in the range 0·05 to 0·15.

CALIBRATION OF THE SPECTROPHOTOMETER—

Evaporate to dryness, in platinum dishes, portions of a standard solution of boron, covering the range 0 to 0·15 μg of boron, on a boiling-water bath in the presence of 2 drops of 10 per cent. w/v sodium hydroxide solution.

Add to the contents of each dish 2·5 ml of zirconium sulphate solution prepared from pure zirconium as described under "Procedure," and then warm the dish gently to dissolve the residue. Carry out the procedure described above, and construct a calibration graph of concentration of boron against net optical density.

RESULTS

The results for the determination of boron in samples of zirconium and zirconium alloys by the method are shown in Table I. Comparison is made with a method involving separation of the boron by distillation as methyl borate. The recoveries quoted are for boron added as a dilute solution of boric acid to the contents of the flask before dissolution of the sample.

DISCUSSION OF THE METHOD

The formation of the boron - curcumin complex in solution demands the fairly rigorous exclusion of water.[7] In order to extend the lower limit of detection of the method described

TABLE I

RESULTS FOR THE DETERMINATION OF BORON BY THE DIRECT METHOD

Sample		Boron added, p.p.m.	Boron found, p.p.m.	Boron recovered, %	Boron found after separation as methyl borate, p.p.m.
Zirconium sponge‡	1	—	<0·2 (Nil)*		
		—	<0·2 (Nil)*		
	2	—	<0·2 (Nil)*		
		—	<0·2 (Nil)*		<0·2
	3	—	<0·2 (Nil)*		
		—	<0·2 (Nil)*		
	1	0·5	0·56	112	
		0·5	0·53	106	
	2	0·5	0·48	96	
		0·5	0·46	92	
	3	0·5	0·47	94	
		0·5	0·51	102	
ATR alloy§	1	—	<0·2 (0·16)†		<0·2
		—	<0·2 (0·16)†		
		—	<0·2 (0·17)†		
		0·5	0·57	82	
		0·5	0·61	90	
		0·5	0·71	110	
	2	—	<0·2 (0·13)†		<0·2
		—	<0·2 (0·11)†		<0·2
		—	<0·2 (0·11)†		
		0·5	0·57	90	0·58
		0·5	0·64	104	
		0·5	0·59	94	
Zircaloy II‖		—	<0·2 (Nil)*		<0·2
		—	<0·2 (Nil)*		
		—	<0·2 (Nil)*		
		—	<0·2 (Nil)*		
		0·5	0·48	96	
		0·5	0·54	108	
		0·5	0·48	96	

* Not significantly different from the reagent blank value.
† Figure given to allow recoveries to be calculated.
‡ Purified by the iodide process.
§ Contains 0·5 per cent. of copper and 0·5 per cent. of molybdenum.
‖ Contains 1·5 per cent. of tin, 0·12 per cent. of iron, 0·05 per cent. of nickel and 0·1 per cent. of chromium.

previously, in which the portion of sample solution taken was only 0·25 ml containing 10 mg of sample, a larger amount of sample must be taken. To do this without increasing the amount of water taken, either the zirconium content of the solution must be increased or the initial dilution must be with a non-aqueous solvent to allow a larger portion to be taken. The latter course was taken to avoid concentration by evaporation, which could, possibly, lead to a loss of boron.

In previous work a mixture of one part of sulphuric acid to three parts of acetic acid had been used as the medium for complex formation. When these conditions were applied in the presence of larger amounts of zirconium (50 mg), a heavy precipitate of zirconium salts formed. In the presence of larger proportions of sulphuric acid, salts did not precipitate. The formation of the boron complex was known to occur over a wide range of sulphuric acid concentrations, but the rates of reaction and subsequent decomposition of the complex, in the concentration of acid necessary to maintain the larger amounts of zirconium in solution, were not known. This was checked by measuring the effect of reaction time on the formation of the complex in 5·5 ml of a mixture of 5 parts of sulphuric acid and 6 parts of acetic acid and containing 62·5 mg of zirconium, as the sulphate, and 300 mg of ammonium sulphate. The results of these experiments (see Fig. 1, p. 474) indicate that the reaction time is not critical at room temperature.

Application of these conditions for forming the complex to zirconium alloys containing tin led to the formation of a flocculent brown precipitate when the reaction mixture was diluted with water before separating the boron complex by filtration. This brown precipitate appeared to be a compound containing curcumin that caused interference with the boron determination. The interfering curcumin compound separated on the filter together with the boron complex and unreacted curcumin. It was subsequently eluted during dissolution of the boron complex in alcohol, to give a solution having some absorption at 555 mμ, the wavelength used for measurement of the boron complex. A brief examination of the properties of the compound formed in the presence of tin showed it to be soluble in moderately concentrated acids, in which the boron complex is insoluble. By adding acid instead of water to the reaction mixture the interfering compound dissolved, and the boron complex could be separated by filtration as before. Washing the residue on the column with acid, before and after removal of the excess of curcumin by extraction with ether, was found to be the most effective method of completely removing the interference. The method described above was found to give complete freedom from interference caused by the presence of tin.

TABLE II

CALIBRATION FOR BORON IN PRESENCE OF PURE ZIRCONIUM

Boron added, μg	Optical density (555 mμ; 4-cm cell)	Net optical density	Optical density per 0·03 μg of boron
0	0·184*	—	—
0·03	0·365	0·181	0·181
0·06	0·550	0·366	0·183
0·09	0·720	0·536	0·179
0·12	0·900	0·716	0·179
0·15	1·090	0·906	0·181

* An optical density slightly higher than the reagent blank value is to be expected, owing to the small boron content of the pure zirconium used.

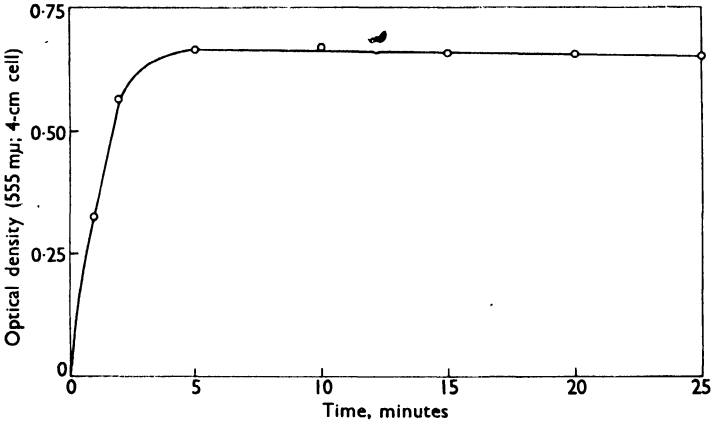

Fig. 1. Formation of the boron - curcumin complex in the presence of zirconium at room temperature; 0·1 μg of boron in a volume of 10 ml

Calibration of the spectrophotometer was carried out in the presence of zirconium in order to avoid any effects arising from the presence of the high salt concentration, although the calibration results obtained (see Table II) indicated that formation of the boron complex was quantitative.

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SHORT PAPERS

Determination of Boron in Zirconium, Hafnium and Titanium

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METHODS described for determining trace amounts of boron, to a maximum specification of 0·5 p.p.m., in zirconium and zirconium alloys[1,2,3] all involve a preliminary separation of boron from the parent metal by distillation of methyl borate.

A study of the boron - curcumin reaction, as described by Hayes and Metcalfe,[3] led us to develop direct procedures for determining large amounts of boron in zirconium and titanium and trace amounts (less than 0·5 p.p.m.) in reactor-grade zirconium, zirconium alloys and hafnium.

After our experimental work had been completed, it came to our notice that Hayes and Metcalfe had continued their studies of the boron - curcumin reaction, and had also developed a direct method for determining extremely small amounts of boron in zirconium. Their procedure is similar to that concurrently and independently developed in our laboratory for determining boron in zirconium, hafnium and titanium.

Because Hayes and Metcalfe's second paper had been accepted for publication in *The Analyst*,[4] and our work had indirectly confirmed the reliability of their procedure, this paper was prepared only to emphasise where our procedure differs from theirs, and also to indicate its application to the determination of boron in hafnium and titanium.

1. DETERMINATION OF TRACE AMOUNTS OF BORON IN ZIRCONIUM, HAFNIUM AND THEIR ALLOYS (0·1 to 0·5 p.p.m.)

(*a*) A 1-g sample is dissolved in a mixture of 5 g of ammonium sulphate and 15 ml of sulphuric acid, sp.gr. 1·84, under reflux as described by Hayes and Metcalfe, but oxidation with nitric acid and boiling under reflux with formic acid is omitted.

(*b*) In Hayes and Metcalfe's "normal method," the main factor limiting the procedure to the determination of boron above 1 p.p.m. is associated with the water present in the aliquot of the sample solution; because the presence of water seriously inhibits the boron - curcumin reaction, the sample aliquot is restricted to 0·25 ml. We have shown that a larger aliquot can be taken, provided the sample solution is diluted with an acetic - sulphuric acid mixture in place of water, and, in this way, it is permissible to take up to at least a 10-ml aliquot. A solution of a 1-g sample is diluted to 50 ml, and the boron - curcumin reaction is applied to a 10-ml aliquot without the need for a preliminary separation of boron from zirconium.

(*c*) The reaction mixture is set aside for 30 minutes. After this time, the reaction is sufficiently complete to permit reproducible results to be obtained; no significant advantage is gained by allowing the reaction to proceed for longer than 30 minutes.

(*d*) Filtration of the reaction slurry as recommended[3] is omitted, and the excess of curcumin is removed more simply by shaking the reaction mixture with ether in a separating funnel (the rosocyanin collects at the interface between the ether and aqueous phases); the bulk of the ether and aqueous phases are discarded before the rosocyanin is dissolved in methanol to a final dilution of 15 ml. (Methanol can be used in place of ethanol without affecting the results.) Optical densities are measured at 5550 Å, as recommended.[3]

(*e*) With these modifications, this direct method can be applied over the range 0·1 to 50 p.p.m., by varying the sample weight and/or volume of sample solution taken.

2. DETERMINATION OF LARGE AMOUNTS OF BORON IN ZIRCONIUM, HAFNIUM AND TITANIUM (Above 50 p.p.m.)

(*a*) Oxidation with nitric acid and boiling under reflux with formic acid (as in 1 (*a*)) is omitted.

(*b*) The sample solution is diluted to a suitable volume with water, and an 0·5-ml aliquot is transferred to a 100-ml calibrated flask. After reaction with curcumin, as described by Hayes and Metcalfe, the mixture is set aside for 30 minutes (see 1 (*c*)); it is then diluted to the mark with methanol. Under these conditions maximum absorption occurred at 5400 Å.

(*c*) The method can be applied over the range 50 p.p.m. to about 1 per cent. of boron in zirconium, zirconium alloys and hafnium. Titanium forms a yellow compound with the curcumin and also retards the reaction between boron and curcumin. Thus in the presence of 0·5 mg of titanium, a standing time of 1 hour is allowed for reaction between boron and curcumin. Increase in the amount of titanium suppresses the reaction further, and the direct determination of boron in titanium is limited to amounts above about 0·1 per cent.

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The Use of Mixed Nitric and Perchloric Acids as a Solvent in the Controlled Oxidation of Cellulose by Boiling Perchloric Acid

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In a previous publication[1] a method was described for the complete oxidation of cellulose in which fuming nitric acid, sp.gr. 1·5, was used as a solvent for cellulose and the solution was added gradually to boiling 72 per cent. perchloric acid. By this means, complete oxidation of cellulose is achieved under controlled conditions, and deflagrations are avoided; deflagrations frequently occur if the cellulose is first oxidised with nitric acid and the solution then treated with perchloric acid and evaporated. It was pointed out that concentrated nitric acid, sp.gr. 1·42, is a less suitable solvent than the stronger acid because it dissolves cellulose only slowly after long heating and with considerable oxidation. However, in contrast with nitric acid, sp.gr. 1·5, ordinary concentrated nitric acid can readily be purified in the laboratory by distillation, and this may sometimes be advantageous for the elimination of trace impurities. As 72 per cent. perchloric acid (which can also be distilled) is a dehydrating agent it was thought that a suitable mixture of this acid with nitric acid, sp.gr. 1·42, might replace fuming nitric acid as a solvent for cellulose. This possibility has been investigated, and a suitable procedure has been developed; it is described later in this paper.

If cellulose is treated with nitric acid, sp.gr. 1·42, containing at least one third of its volume of 72 per cent. perchloric acid a gelatinous mass is produced; on heating the mixture the viscosity falls, and at about 65° to 70° C a mobile solution is obtained that can be added to boiling 72 per cent. perchloric acid, as previously described,[1] to oxidise the organic matter. At 70° to 75° C oxidation of organic matter by nitric acid begins, and this reaction, if unchecked, proceeds vigorously with evolution of nitrous fumes; the reaction can, however, be stopped by cooling the beaker in water. It is possible to allow the nitric acid oxidation reaction to proceed as far as it will go and then to complete the oxidation by adding the solution to boiling perchloric acid. There are two objections to this procedure. First, the perchloric acid has then to deal with only a relatively small amount of organic matter, and the reaction therefore liberates so little heat that strong outside heating is necessary to remove nitric acid and water and to maintain the perchloric acid at its maximum concentration. Second, extremely little perchloric acid is consumed, and the large excess has to be evaporated. There is, therefore, considerable advantage to be gained in preventing the nitric acid reaction, thus ensuring that perchloric acid is the essential oxidant in the process.

It has been found that the start of the nitric acid - cellulose reaction is delayed by the presence of perchloric acid and that it is therefore easier to dissolve cellulose in mixtures rich in perchloric acid; a possible reason for this will be mentioned later. However, perchloric acid in excess of that required to oxidise cellulose must be evaporated, and, as this increases the time needed to complete the process, a compromise concentration of acid has been adopted. The amounts recommended are 7 ml of nitric acid, sp.gr. 1·42, and 3 ml of 72 per cent. perchloric acid per gram of cellulose; the composition of the mixed acid may be varied between 7·5 to 2·5 and 6·5 to 3·5 to suit particular experimental requirements. Under these conditions cellulose can be dissolved completely at 60° to 70° C with only slight oxidation by nitric acid, and this reaction can easily be stopped by cooling the beaker in water.

It was considered desirable to ensure that the reaction between nitric acid and cellulose could not become dangerous; accordingly, experiments were carried out in which 1- to 10-g portions of cellulose were heated with nitric - perchloric acid until the reaction began; the reaction was then allowed to proceed as far as it would go. These experiments were not confined to the conditions recommended above; the ratio of perchloric acid to cellulose was varied and, at each ratio, experiments were carried out with different ratios of nitric to perchloric acid. The results of 24 such experiments are summarised in Table I in order of decreasing vigour of reaction, this being assessed arbitrarily as violent, very vigorous, vigorous and slow. Obviously, as a subjective judgment is involved, overlap of the four categories occurs, but the Table affords some guide as to the behaviour of cellulose when heated with various mixtures of nitric acid and perchloric acids. No untoward incident occurred in these experiments, and no liquid was lost from the beaker even in those reactions assessed as "violent."

TABLE I

EFFECT OF AMOUNT AND COMPOSITION OF MIXED ACID ON THE REACTION BETWEEN NITRIC ACID AND CELLULOSE

Vigour of reaction	Volume of mixed acid per g of cellulose, ml	Composition of mixed acid, ml of nitric acid, sp.gr. 1·42, per ml of 72 per cent. perchloric acid
Violent	4·0 to 5·5	1·2 to 3·0
Very vigorous	6·0 to 8·0	2·0 to 3·0
Vigorous	8·0 to 10·0	1·8 to 3·5
Slow	5·0 to 6·25	4·0

In general, the vigour of the reaction depends mainly on the ratio of the volume of mixed acid to weight of cellulose, and it is this ratio, therefore, that is indicated in column 2 of Table I instead of the perchloric acid to cellulose ratio, which was one of the independent variables in the experiments. The composition of the mixed acids has rather less effect at nitric to perchloric acid ratios below about 3·5 to 1, though if the ratio is above this value the reaction proceeds only slowly.

The recommended conditions for dissolving cellulose correspond to the third line of the Table; hence, if the reaction is allowed to proceed it will do so vigorously but without violence and can easily be stopped at any time by cooling the beaker in water. Further, a violent reaction can only be produced as the result of gross deviations from the recommended conditions.

An incident that occurred in one experiment is of interest. Ten grams of cellulose were dissolved in a mixture of 45 ml of nitric acid and 30 ml of perchloric acid, and the temperature rose to 75° C. The nitric acid reaction began and, after proceeding some way, was stopped by cooling the beaker in water. At this stage a considerable amount of white granular solid separated out; the acid was poured off, and water was added to the residue, which dissolved with evolution of nitrous fumes and the production of a transient blue colour. The precipitate appears to have been the hydrate of nitrosyl perchlorate,[2] $(NO)ClO_4.H_2O$, which has been prepared by passing a mixture of nitric oxide and nitrogen dioxide through 72 per cent. perchloric acid. In the above experiment the nitrogen oxides were generated *in situ*.

It is suggested that the formation of nitrosyl perchlorate may explain the effect of perchloric acid in delaying the start of the reaction between nitric acid and cellulose. Nitric acid oxidation reactions are autocatalysed by the nitrous acid (or lower oxides of nitrogen) produced therein and substances, such as urea, sulphuric acid and hydrazine that destroy nitrous acid are frequently added as stabilisers to nitric acid solutions containing reducing agents. For example, the oxidation of ferrous ion by dilute nitric acid is avoided by the presence of sulphamic acid. In the experiments described in this paper the removal of nitrous acid or NO_2 by such reactions as—

$$HNO_2 + HClO_4 \longrightarrow (NO)ClO_4 + H_2O$$

$$\text{or } 2NO_2 + HClO_4 \longrightarrow (NO)ClO_4 + HNO_3$$

would be expected to delay the start of the reaction between nitric acid and cellulose.

METHOD

Before using perchloric acid, the analyst should familiarise himself with its properties and the necessary precautions to be taken in handling it. In this connection, the report[3] of the Analytical Methods Committee should be consulted, and particular care should be taken to ensure that fume cupboards are of suitable construction. The usual precautions should also be taken for handling hot concentrated acids, including suitable shielding for the face.

PROCEDURE—

The procedure described below is suitable for 10 g of cellulose; for smaller amounts, use proportionately smaller amounts of reagents. A larger amount is best dealt with by dividing it into portions not exceeding 10 g and treating these separately. Apart from permissible variations mentioned in the last paragraph below, amounts of reagents should be rigidly adhered to.

Transfer 10 g of cellulose to a 250-ml beaker, and add a mixture of 30 ml of 72 per cent. perchloric acid and 70 ml of concentrated nitric acid, sp.gr. 1·42. The nitric acid should be reasonably free from lower oxides of nitrogen, *i.e.*, colourless or extremely pale yellow. Warm under a lamp or on a water-bath to a temperature not exceeding 65° to 70° C, swirling the beaker occasionally to mix the contents, until the solution is sufficiently mobile to be handled with a transfer pipette. At this stage some oxidation may occur with darkening of the solution and evolution of oxides of nitrogen. If this reaction is allowed to proceed, a considerable proportion of the organic matter will be oxidised by the nitric acid, and much less perchloric acid will be needed later. The solution will therefore contain excess of perchloric acid, and the procedure will be unduly lengthened by the extra time needed for evaporation. To avoid this, the nitric acid reaction should be stopped by cooling the beaker in running water to below 40° C.

By means of a transfer pipette, add the acid solution of cellulose, in small portions, to 5 ml of boiling 72 per cent. perchloric acid in a 50-ml beaker covered as far as possible with a clock-glass; vigorous oxidation of organic matter occurs, and yellow fumes of chlorine are evolved. After each addition, wait for a few seconds until the yellow fumes inside the beaker have been completely replaced by white perchloric acid vapour before adding more cellulose solution. This is essential to ensure that the maximum concentration of perchloric acid is maintained. (WARNING —At no stage in the procedure should nitrous fumes be evolved; these indicate dilution of the perchloric acid, perhaps through inadequate heating, and therefore incomplete oxidation. In such an event, discontinue addition of the cellulose solution until perchloric acid vapour is again evolved freely.)

When all the cellulose solution has been added, rinse the 250-ml beaker once or twice with a little nitric acid, and add the rinsings to the perchloric acid. Evaporate the perchloric acid solution as much as is necessary for the subsequent treatment.

During the procedure the volume of perchloric acid in the 50-ml beaker may decrease or increase according to the rate of heating. In the former instance it may be necessary to add more acid to replace losses; if so, ensure that the acid is again boiling before continuing with the oxidation. If there is a tendency to change in the volume of perchloric acid, control can be exercised by varying the nitric acid - perchloric acid volume ratio in the solvent between the limits 65 to 35 and 75 to 25. The original volume of boiling perchloric acid and its rate of heating may also be adjusted. Ideal conditions are those in which the volume of perchloric acid remains at about 5 ml throughout the procedure.

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Determination of Trace Amounts of Chloride in Sodium Bromide

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SEVERAL procedures for determining small amounts (of the order 0·2 to 2 per cent.) of chloride in bromides have been described in the literature. Most of these are based on selective oxidation of bromide, although Rieman and Lindenbaum[1] have used ion-exchange chromatography to separate the two ions. Many different oxidising agents have been employed, *e.g.*, ceric sulphate,[2] potassium dichromate,[3] nitric acid,[4] telluric acid,[5] potassium permanganate[6,7] and potassium bromate.[8,9,10] In most of these methods complete removal of the bromide was attempted. Haslam,[7] however, used a permanganate oxidation to remove the bulk of the bromide and then determined the chloride by differential potentiometric titration. None of these procedures was sufficiently sensitive for application to samples containing 100 to 500 p.p.m. of chloride, and the investigation described here was directed towards the development of a method for determining concentrations of this order in sodium bromide.

The most suitable reagent for the selective oxidation of bromide in the presence of chloride appeared to be potassium bromate in the presence of nitric acid. The analytical-reagent grades of both these reagents have extremely low chloride contents. Potassium bromate has been used by Andrews[8] and later by Berg[9] and by D'Ans and Hofer.[10] The latter authors made a fairly detailed study of oxidation conditions and concluded that a pH of about 2 was the most suitable for determining small amounts of the order of 0·2 per cent. of chloride. They used aluminium nitrate to control the pH. In our investigation this was found to be unnecessary.

The oxidation was carried out in dilute solution (2·5 g of sodium bromide in 150 ml of water) so as to minimise the danger of over-oxidation. In the first experiments the residual bromide and the chloride were then determined potentiometrically: the residual bromide was found to be equivalent to about 0·2 ml of 0·01 N silver nitrate. However, because the end-point for chloride was poor under these conditions, the results were not particularly accurate or reproducible. In an effort to improve the chloride end-point the addition of a number of organic solvents was tried. Methanol, ethanol and ethylene glycol all produced an improvement in the chloride end-point when added, at the 50 per cent. level, to solutions containing chloride and bromide ions at the same concentrations as those in the test solutions. Acetone was less satisfactory. All these solvents had an adverse effect on the bromide end-point and gave unsatisfactory results when added to actual test solutions.

In 50 per cent. acetic acid, however, both the chloride and bromide end-points were extremely sharp. Further, it was observed that when an equal volume of acetic acid was added to an oxidised sodium bromide solution an end-point for only chloride was obtained. This suggested that the residual bromide in the solution was being rapidly oxidised in the cold.

To test this hypothesis three solutions were prepared containing 0·24 g of potassium bromate in 200 ml of water to which sufficient nitric acid had been added to make the pH 0·9, this being the pH of an oxidised solution of sodium bromide after the addition of acetic acid. To each of these solutions were added 2 ml of 0·01 N sodium bromide. The solutions were set aside for 5, 15 and 45 minutes, respectively, and then titrated with 0·01 N silver nitrate. The amount of bromide remaining in the solution that had been set aside for 5 minutes was equivalent to 1·4 ml of 0·01 N silver nitrate, and in the solution set aside for 45 minutes the amount remaining was still equivalent to 0·6 ml of 0·01 N silver nitrate. The experiment was repeated, with the potassium bromate being dissolved in 50 per cent. aqueous acetic acid (pH 1·35). The reaction rate in this medium appeared to be similar to that in nitric acid, the amount of bromide remaining in the solution set aside for 45 minutes being equivalent to 0·65 ml of 0·01 N silver nitrate.

Finally, the experiment was repeated with 50 per cent. aqueous acetic acid being used to which sufficient nitric acid had been added to lower the pH to 0·9. The amount of bromide remaining in the solution set aside for 5 minutes was equivalent to only about 0·15 ml of 0·01 N silver nitrate; after 15 minutes the amount was about 0·1 ml, whereas after 45 minutes no bromide at all could be detected. When a smaller amount of 0·01 N sodium bromide (0·2 ml) was used no bromide could be detected in solutions set aside for 5 minutes. The reason for this greatly accelerated rate of reaction is not clear. When a similar experiment was carried out with 2 ml of 0·01 N sodium chloride the concentration was unchanged after 45 minutes.

Comparative oxidations on solutions containing (*a*) bromide alone and (*b*) bromide *plus* chloride showed that the mixed halides appeared to be without effect on one another in the oxidation.

As well as increasing the accuracy of the method, the removal of all the bromide allowed the sensitive and accurate "dead-stop" end-point procedure of Price and Coe[11] to be used to determine the chloride.

The concentration of nitric acid used in the hot oxidation must not be too low, otherwise the amount of bromide remaining might be too great to be oxidised in the cold except on prolonged standing.

At levels of chloride below about 100 p.p.m. results tend to be high, suggesting that small amounts of bromide might have remained unoxidised. By using a ring oven technique[12] sensitive to 5 μg of bromide, it was established that the solutions, after oxidation, contained less than 5 μg of bromide. By using a smaller initial volume for the hot oxidation the results obtained were nearer the theoretical, but it would be preferable to use a larger sample at these extremely low levels.

To test the method, chloride-free sodium bromide was required and was prepared as described below. Reagent-grade sodium bromide (50 g) and potassium bromate (21 g) were dissolved in 800 ml of water, and 300 ml of 2 M nitric acid were added. The bromine evolved was absorbed in analytical-reagent grade ammonia solution, sp.gr. 0·880; the excess of ammonia was boiled off and the ammonium bromide decomposed by boiling with an equivalent amount of sodium hydroxide prepared from sodium metal.

METHOD

REAGENTS—

Potassium bromate solution—Dissolve 11·5 g of analytical-reagent grade potassium bromate in 250 ml of water.

Nitric acid, 2 M—Dilute 127·5 ml of analytical-reagent grade concentrated nitric acid to 1 litre with water.

Glacial acetic acid—Analytical-reagent grade.

Silver nitrate solution, 0·01 N.

PROCEDURE—

Weigh accurately about 2·5 g of the sample of sodium bromide into a 1-litre beaker. Add 125 ml of water and 25 ml of potassium bromate solution and then 15·0 ml of 2 M nitric acid. Pass a vigorous stream of filtered air through the solution, and heat on a steam-bath, keeping the temperature of the solution at 65° to 75° C. After 30 minutes add a further 2·0 ml of 2 M nitric acid, and continue heating for a further 15 minutes. Cool to room temperature, transfer the contents to a 250-ml beaker, and add 100 ml of glacial acetic acid. Stir for 15 minutes with a mechanical or electrical stirrer, and titrate with 0·01 N silver nitrate solution in the manner described by Price and Coe.[11]

RESULTS

Known amounts of chloride were added to solutions of sodium bromide, and the chloride was determined by the procedure described above. The results shown in Table I indicate that excellent results are obtained for between 140 and 420 p.p.m. of chloride; at levels between 36 and 110 p.p.m. the values obtained were high by about 10 per cent. The standard deviation at a level of 142 p.p.m. was ±4·3 p.p.m. of chloride.

TABLE I

RECOVERY OF CHLORIDE IN PRESENCE OF SODIUM BROMIDE

Chloride added, p.p.m.	Chloride found, p.p.m.	Number of analyses	Recovery, %
0	0	1	—
36	40*	2	111
71	76*	2	107
107	117	2	109
142	141	8	99
213	215	4	101
284	288	4	101
355	352	2	99
426	420	2	99

* In these determinations the total volume of reactants at the start of the hot oxidation was 115 ml instead of 165 ml.

The proposed method has been applied to some special grades of commercial sodium bromide known to have a low chloride content. No difficulties were experienced in any of the analyses, and the results shown below were reproducible.

Sample number	1	2	3	4	5	6	7	8	9	10
Chloride content, p.p.m.	387, 398	190, 190	103, 101	72, 71	378, 365	108, 98	102, 112	98, 94	132, 122	201, 213

The standard error of a single determination was ±6 p.p.m. of chloride.

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The Spectrographic Determination of Trace Impurities in Arsenic

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An adaption is described of the procedure used for the spectrographic analysis of gallium arsenide,[1] in which 1 g of gallium arsenide was treated with hydrochloric acid, carbon tetrachloride and bromine, the arsenic removed by evaporation and the gallium extracted as the chloride by isopropyl ether in the presence of hydrochloric acid. The impurities remaining in the acid phase were concentrated to small bulk in the presence of copper sulphate and sulphuric acid, transferred to a carbon electrode and excited in a d.c. arc.

The same procedure was applied to arsenic metal, except that the extraction with isopropyl ether was omitted. It was found that recoveries of zinc, cadmium, lead, aluminium, nickel, vanadium, titanium and cobalt were low and variable (<50 per cent.). It was suspected that this was due to loss by volatilisation of the chlorides of these elements during the evaporation to near dryness after the initial treatment with hydrochloric acid, carbon tetrachloride and bromine. As originally applied to gallium arsenide, the arsenic was removed in the presence of about 0·5 g of gallium, and this would have the effect of minimising the loss of trace elements during evaporation. In a previous investigation it had been shown that up to 0·3 mg of gallium did not interfere with the spectrographic finish.[2] Further experiments were therefore carried out in which 0·25 mg of gallium, as chloride, was added to the initial reaction mixture, and this was found to be sufficient to hold back the trace elements. The final procedure adopted is described below.

PROCEDURE—

An approximately 1-g sample of arsenic metal, pre-etched with pure hydrochloric acid (for purification of reagents see Oldfield and Mack[1]), was weighed into a clean polytetrafluoroethylene beaker. To the sample were added, successively, 5 ml of pure hydrochloric acid, 5 ml of carbon tetrachloride, 2 ml of bromine and 1 ml of gallium chloride solution (containing 250 µg of gallium). A reagent blank solution was taken through the procedure simultaneously. After the sample had dissolved the solution was evaporated to small bulk to remove all the arsenic; 1 ml of 0·1 N sulphuric acid and 1 ml of copper sulphate internal standard solution were then added, and the mixture was again evaporated to small bulk (≃ 0·2 ml). The solution was then transferred to a pre-arced carbon electrode and analysed spectrographically by the original procedure.[1]

RECOVERY OF IMPURITIES—

Samples of arsenic to which had been added 0·5-, 1·0- and 2·0-μg amounts of the trace elements were analysed alongside samples with no additions, and the recoveries were determined by deducting the results for the untreated samples from those for the treated samples. Recoveries of silicon, magnesium, manganese, chromium, bismuth, aluminium, beryllium, vanadium, zirconium, cobalt, molybdenum, titanium, indium, cadmium and nickel were better than 80 per cent. The recovery of zinc was low and that of lead unusually high.

Application of the method showed blank values of the same order as those obtained previously,[1] as were the sensitivity and reproducibility values.

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1. Oldfield, J. H., and Mack, D. L., *Analyst*, 1962, **87**, 778.
2. Oldfield, J. H., and Bridge, E. P., *Ibid.*, 1961, **86**, 267.

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Book Reviews

CHEMICAL ANALYSIS: THE WORKING TOOLS. Volumes I, II and III. Edited by C. R. N. STROUTS, H. N. WILSON and R. T. PARRY-JONES, with the assistance of J. H. GILFILLAN. Second Edition. Pp. xx + 467 (Volume I); xii + 479 (Volume II); xii + 273 (Volume III). Oxford University Press. 1962. Price (3 Volumes) £7 7s.

The first edition of this book was published in 1955 under a slightly different title, "Analytical Chemistry—The Working Tools," and it was in two volumes. The second edition has been revised and extended to include a third volume. In addition, there has been a considerable rearrangement of chapters to give a more logical presentation.

The volumes are not separately named, but Volume I deals with the more basic operations of the chemical analyst. In this edition the opportunity has been taken to include a new chapter on vacuum fusion and vacuum extraction methods for the determination of gases in metals, although it is hardly possible to do full justice to this topic in 14 pages. There is also a new chapter on titration in non-aqueous solution and the chapter on chromatography now includes a section on gas chromatography. In another new chapter, headed Miscellaneous Techniques, there are sections on refractive index, differential refractometry and isotope-dilution methods.

In Volume II there are chapters on the determination of pH, electrometric titration, conductometric analysis, polarography, electro-deposition, four chapters on colorimetric analysis and absorption spectrophotometry, emission spectrography, including flame photometry and atomic-absorption spectrophotometry, X-ray spectrochemical analysis and X-ray diffraction analysis. Again, it is hardly possible to deal adequately with some of these techniques within the space of a single chapter, but one has to remember that it was the purpose of I.C.I. Ltd. to present in the first edition an account of the working tools used in their laboratories. In this, the second edition, we can see that as a result of the rapid development of instrumental techniques many are now included as routine working tools of the analyst.

The third volume has been given over entirely to the quantitative examination of organic substances, in particular to the determination of elements and certain functional groups. There is also a chapter on the determination of molecular weight.

It is not possible to comment in any detail on such a comprehensive work. There is much to praise and little to criticise. The reviewer cannot do better than to quote from his review (in another place) of the first edition—"that the analysts of I.C.I. should have painted on such a broad canvas is the clearest evidence of the expanding frontiers of analysis as an art and science. The book will be of the greatest value to all analysts; if it serves also to convince young graduates that a worth-while career is open to those of them who can shoulder the analytical discipline, Imperial Chemical Industries will have done a great service." It only remains to add that at seven guineas for the three volumes, beautifully produced by the Clarendon Press, I.C.I. has done a further service, as have the editors and the many members of the staff of the Company who have contributed.

R. C. CHIRNSIDE

DÜNNSCHICHT-CHROMATOGRAPHIE: EIN LABORATORIUMSHANDBUCH. By H. R. BOLLIGER, M. BRENNER, H. GÄNSHIRT, H. K. MANGOLD, H. SEILER, EGON STAHL and D. WALDI. Edited by EGON STAHL. Pp. xvi + 534. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 56.

Paper chromatography arrived with a flurry of improvisation, but many chemists first heard of thin-layer chromatography from the advertisements of firms selling materials and tools for the job. These inducements have been followed quickly by the book under review and another, both published (in German) in 1962. The commercial enterprise informing this effort is praiseworthy, but drain-pipes and filter-paper still have advantages; the basic kit for thin-layer chromatography cost £70 when it was launched on to the British market, and this book sells at £5. The book is indeed directed at chemists nurtured on golden handshakes rather than at those eking out puffs of the cold wind of charity; the early pages are resplendent in pictures and details of magnificent pieces of commercial apparatus, but the possessors of a £50 tool should not need to buy a book for instruction on washing it. This air of opulence is a pity, because a handyman can easily make the essentials for a method that bids fair to supplant and supplement less handy procedures and to reduce the need for experiments on animals. "*Dünnschicht Chromatographie*" proclaims the versatility of the method, and will interest all types of users and potential users; English chemists should heed its message and reserve their canniness for the elaborations.

The book has a wider scope than the title implies. Some of the chapters review all the methods for separating mixtures of members of a class of natural products, with the emphasis on thin-layer chromatography. In places, results fail to keep pace with the narrative, so that procedures used in paper chromatography can be recommended as no more than eligible for application to chromatoplates. Nevertheless, the chapters abound in authoritative information, and the details are always enough to give the beginner a good start. Thin-layer chromatography excels in speed and sharpness of separation, and in the inertness of the supports; corrosive spray reagents may be used, and material in the spots may be determined, with little interference, by chemical methods or by volatilising the fraction into apparatus for gas chromatography or mass spectrometry. It lends itself more aptly than paper chromatography to quantitative analysis.

The first quarter of the book deals in great detail with the apparatus, materials, procedures, skills and theory of thin-layer chromatography. Quantitative methods are discussed. The chapter on theory refutes the view that paper and thin-layer chromatography are, respectively, partition and adsorption processes, since the thin-layers are supports that can function as weak ion-exchangers and, accordingly, perform well in the chromatography of polar compounds and inorganic ions.

The rest of the book discusses separations of classes of compound. The chapter (by Mangold) on aliphatic lipids emphasises the rise in importance of gas and thin-layer chromatography, and illustrates the usefulness of methods with reversed phases and with layers containing complexing agents.

Stahl and Jork trace methods of thin-layer chromatography from their beginnings in terpene chemistry to recent developments in that series. Chapters on vitamins (Bolliger), steroids (Waldi) and organic bases (Waldi and Stahl) illustrate the scope of the method and introduce, without recommendation, a procedure involving loosely strewn horizontal layers of the adsorbents normally used for column chromatography. Doubts are aroused that preparative chromatography might be conducted better with adsorbents sandwiched between plates than in columns.

Gänshirt reviews the thin-layer chromatography of drugs, and Waldi examines clinical and pharmacological applications. These chapters and the next overlap some of the earlier topics, but they will interest pharmacists, analysts, and forensic chemists. Methods are recommended, for instance, as tests for pregnancy and for the estimation of alcohols in blood. The succeeding chapter on the surveillance of bulk chemicals will remind industrial chemists that a method eminent in microanalysis is still in place in the production department.

In separations of amino acids, peptides, nucleic acids, nucleotides and sugars thin-layer chromatography lays siege to the fastnesses of paper chromatography; the reviews on these topics make a strong case for the speed and efficiency of the new method, especially now that thin-layer electrophoresis and ion-exchange chromatography have been furnished with materials and feasible methods. The analysis of sugars need no longer suffer the limitations of a cellulosic chromatographic support.

Seiler gives an account of the separation of inorganic cations and anions, for which layers of specially purified materials are needed. The book ends with the Merck list of spray reagents and a glossary of the jargon of the subject, with English and French equivalents. The list omits some useful reagents and receives short shrift from the index, but it is still of help to all practitioners of chromatography.

The book is stoutly bound and well printed. Mistakes are few and the German text translates easily (an English version is under consideration). It can be recommended to libraries, since it covers literature, much of it rare in this country, that has escaped comprehensive review in English. The price is high, and the publishers should, in future editions, avoid features that mar this one: the paper glares in artificial light; placing of references at the end of each chapter confuses readers, unless each page carries a note of the reference-page; the index is scanty (for example, Bromcyan occurs in the text, and is listed under "Königs Reagenz," but neither entry appears in the index).

Publishers and advertisers would help chemists by issuing abstracts and announcements on chromatography as a file kept frequently up to date. Such a system would ease pressure on the journals and spare librarians the burden of buying books, such as this, that soon become dated in topics with rapidly extending applications. A. G. LONG

GAS CHROMATOGRAPHIE 1961. Lectures and Discussions presented at the 3rd Symposium on Gas Chromatography held at VEB Chemische Werke Buna, Schkopau, Germany, May 16th to 19th, 1961. Edited by MANFRED SCHRÖTER and KLAUS METZNER. Pp. viii + 528. Berlin: Akademie-Verlag. 1962. Price DM 46.

The proceedings of the 3rd Symposium on Gas Chromatography of the Working Party on Gas Chromatography of the German Democratic Republic are presented in a series of 35 papers together with the relevant discussions in 528 pages of well-printed text, all in German. Unfortunately, the binding is altogether too flimsy; it did not stand up to the vagaries of the postal system in January.

The first point to note is that there is a nice balance between theoretical considerations, new apparatus and practical applications of gas chromatography. Moreover, the papers are not presented in separate sections.

On the theoretical side, a great deal of attention (108 pages) is paid to the geometric and chemical modification of adsorbents for gas chromatography, and there are contributions on the influence of gas chromatography on sorption research and on partition chromatography on adsorbed layers. Other theoretical papers deal with such subjects as the Taylor - Golay effect and eddy diffusion, adsorption isotherms and problems relating to elution.

The papers on apparatus present a lot of new and useful ideas. They deal with such varied topics as electromechanical integrators, the measurement of small currents, new developments in gas-chromatographic apparatus and differential integral amplifiers. There is an interesting contribution by British authors Evans and Gittins on gas-chromatographic separations with equipment of high sensitivity. Separations achieved on glass spheres are described, as are methods of evaluation of capillary columns.

Analytical chemists, and particularly those concerned with organic separations, will get some useful ideas from the more practical papers. Questions arise, such as: shall we be able, as is suggested in one paper, to carry out group identification of so called "non-volatile aromatic substances" by pyrolysis with and without catalyst and examination of the products for H_2, CO, CH_4 and CO_2.

Will the method recommended by one author for the determination of small amounts of certain organic substances in dilute aqueous solution, *viz.*, separation on polyethylene glycol and then combustion of the separated substance to CO_2 and automatic titration of the resulting carbonic acid, be a valid one.

Again, what is to be our attitude towards reaction chromatography in which the constituents to be separated are first converted to more readily separated substances by passage over appropriate reactor fillings.

Although the gas chromatographic method may be vastly superior to the methods of analysing petrol previously employed, is this a better method of assessment of a petrol than a performance test in a motor.

The analytical chemist in the polymer industry will find a great deal of interest, including observations on the examination of divinyl benzene, a cross-linking agent for ion-exchange resin production. Moreover, he will note that some useful information is provided on the determination of trace impurities in styrene and acrylonitrile monomers as well as the automatic process control of the determinations of methane, ethane, propane and acetylene in ethylene before polymerisation.

Other process controls discussed are those dealing with the production of octanol by hydrogenation of unsaturated C_8 aldehydes, and the regulation of distillation columns.

Among the miscellaneous topics we may mention the use of neon as a carrier gas, the determinations of helium and hydrogen in natural gas, preparative chromatography, degrees of impregnation of stationary phases, the examination of mixed monohydric phenols, respiratory problems, the separation of paraffins up to C_{32} and methyl esters up to C_{26}, and, further, work on the pyrolysis of organic substances by a British author Jones.

This book contains a lot of useful information. Besides the papers, there are some revealing contributions in the discussions. Enthusiastic gas chromatographers, if that be the term, should read this book, polish up their German in the process, and capture some new ideas.

JOHN HASLAM

THE U.S. FEDERAL FOOD, DRUG AND COSMETIC ACT (JUDICIAL AND ADMINISTRATIVE RECORD), 1958–1960. By VINCENT A. KLEINFELD and ALAN H. KAPLAN. Pp. 509. Washington 4, D.C.: Commerce Clearing House Inc. Price $17.50.

Members should be grateful to the Food Law Institute of New York for sponsoring (at a cost of some 3000 dollars) the publication of this, the fifth volume in the Institute's series on the F.D. and C. Act, which now covers the whole of the modern period from 1938 to 1960. Remembering that the U.S. legislation covers cosmetics and also animal foods, as is not yet the case in this country, the publication of this authoritative work is a timely reminder of the pace at which changes are developing the world over in food and drugs legislation. The major changes of legislation covered by this volume arise, as may well have been expected, from the steps taken by the Administration to deal with chemical additives and food colours. Clearly, British exporters of food and drug products to the U.S. will be well advised to have a copy of Kleinfeld and Kaplan in their reference libraries. It is obviously more economical to know the law rather than to run into trouble through ignorance.

Basically, this volume is compiled as a work of reference for attorneys advising these industries, and its first section of over 200 pages gives the full text of legal opinions in cases heard during 1958–60 in District Courts, in the Court of Appeal and in the Supreme Court of the U.S. It is somewhat surprising that so many High Court hearings proceed from the administration of the law in this field, though it must be remembered that all F.D.A. proceedings go to higher courts. To our readers these reports may well make interesting bedside reading, though they may have little application to legal problems in this country. They certainly show what thoroughness, energy and resources the Administration brings to its duties.

The second section of the work reprints "Statements of General Policy or Interpretation" issued by the F.D.A. during 1958–60. These are somewhat reminiscent of the activities of the Advisory Service instituted here by the Ministry of Food after the last war, but now unfortunately abandoned. They illustrate the importance the F.D.A. attaches to official advisory and educational work on the application of this legislation to individual traders and to particular problems in the presentation of their products to the consumer, and is of considerable interest to both the lawyer and to the technical man. Many issues handled in this section are of sufficient interest to repay careful study by U.K. chemists.,

A third section contains all the new regulations that have been given the force and effect of law and promulgated during 1958–60 dealing with definitions and standards of identity of food. It should be of much value to U.K. food exporters to find the whole of this information available in one volume.

The final major part of the work furnishes references to the pertinent material for the period 1958–60 in connection with any problem that may arise under any section of the Act. This is primarily the lawyer's field, but it is worth noticing that the F.D.C. Act, as amended to 1960, is here given in full, as are also the Food Additives Amendments of 1958, the Colour Amendments of 1960 and Regulations relative to New Drugs.

This cannot be the end of the story of the energy with which the F.D.A. has tackled these pressing problems of consumer protection, and we expect that the Food Law Institute will have to sponsor yet another volume in this series in the not too distant future. Again, we congratulate the Institute on its disinterested service to the public, and we hope that British firms may be persuaded to support the good work.

C. A. ADAMS

ANALYTICAL STANDARDS FOR TRACE ELEMENTS IN PETROLEUM PRODUCTS. By H. S. ISBELL, R. STUART TIPSON, J. L. HAGUE, B. F. SCRIBNER, W. HAROLD SMITH, CLARENCE W. R. WADE and ALEX COHEN. National Bureau of Standards Monograph 54. Pp. iv + 23. Washington, D.C.: Superintendent of Documents, U.S. Government Printing Office. 1962. Price 25 cents.

The provision of standard compounds to check the accuracy of analytical methods is particularly difficult in the determination of trace elements in petroleum, because of the insolubility of so many compounds in hydrocarbon media. The American Petroleum Institute Committee on Analytical Research therefore invited the National Bureau of Standards to develop a series of stable (preferably solid) oil-soluble compounds for use as analytical standards.

This publication gives a clear account of the work. Having outlined the problem, the authors describe the work done in selecting suitable compounds, their preparation and purification, chemical and spectrographic analysis, and finally the methods employed for dissolution in petroleum oils.

Although 24 out of the 28 standards selected have been available for some 2 years, full details of the reasons for selecting these 28 from over 150 considered, and their methods of preparation, have not previously been published.

The standards comprise compounds containing aluminium, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, mercury, nickel, phosphorus, potassium, silicon, silver, sodium, strontium, zinc, tin and vanadium as the element of interest. Each of these compounds can be used to prepare a stable reference oil solution, thus providing the analyst with a set of reliable bench standards containing traces of the required element. Further, each of the solutions is compatible with any other, so that standards containing several elements can be readily blended (a data sheet is supplied with each compound).

This publication, therefore, provides the analyst, and in particular, the petroleum specialist, with an appreciation of the work involved in preparing accurate standards for trace determinations, and also working instructions for making standard oil solutions for both chemical and instrumental methods of analysis.

MISS J. P. DIXON

RESIDUE REVIEWS: RESIDUES OF PESTICIDES AND OTHER FOREIGN CHEMICALS IN FOODS AND FEEDS. Volume 1. Edited by FRANCIS A. GUNTHER. Pp. iv + 162. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 22.

The declared object of "Residue Reviews" is to make available on an international basis concise critical reviews of advances in the field of residues in foodstuffs, whether in the understanding of the significance of these residues, in methods for their analysis or in methods for the control of their incidence (by legislation or otherwise). The editiorial advisory board includes members from Belgium, Canada, Denmark, France, Germany, Italy, the Netherlands, Switzerland, the U.K., U.S.A. and the U.S.S.R. This volume is composed of nine chapters, all written in English: French and German are other admissible languages.

Dr. Simone Dormal and Dr. H. Hurtig discuss principles for the establishment of pesticide residue tolerances (11 pp.), setting out briefly the measures at present adopted in the U.S.A., Canada, the United Kingdom, the Netherlands, Switzerland and the U.S.S.R. (in an editorial note fuller accounts for these and other countries are promised in later volumes) and the factors that should be taken into account when reconciling differences for the furtherance of international trade. Dr. Bernard L. Oser writes on the rationale and the legal and other interpretation of experimentally induced cancer by pesticides and food additives (10 pp.). Two chapters deal with physical surface properties. Professor W. M. Hoskins deals with the nature and persistence of pesticide deposits, applied as dusts, granules, emulsion or suspension sprays, on surfaces of all kinds, and with the uptake of toxicants from the deposits (26 pp.). Professor A. S. Crafts and Dr. C. L. Foy write on the physical structure and chemical nature of plant surfaces in relation to the use of pesticides and their formulations (28 pp.). Contributions on residue analysis are made by Dr. D. MacDougall (fluorometric methods, 13 pp.), C. C. Cassil (microcoulometric gas chromatography, 28 pp.) and Dr. D. J. Lisk (oxygen flask combustion, 7 pp.). These summaries, albeit of work much of which has recently been presented elsewhere, are most useful in presenting the capabilities and limitations of these methods of residue analysis at present. Fluorometric methods, when they are applicable, are extremely sensitive, with background interference the main limitation. Microcoulometric gas chromatography is also an extremely sensitive method for either chlorine- or sulphur-containing residues and is capable of a fair measure of specificity.

Oxygen flask combustion followed by spectrophotometric or other end-method is rapid but non-specific; it has been applied to residues containing chlorine, bromine, mercury, selenium, arsenic, manganese and nickel. The remaining chapters are on the effects of pesticides on the flavour and quality of fruits and vegetables by Dr. Charles H. Mahoney (13 pp.); and on the incidence in olive oil of residues resulting from *Dacus oleae* control by Professor M. E. Alessandrini (19 pp.), who describes the successful programme evolved in Italy and the analytical, toxicological and other problems encountered. Dr. Mahoney summarises the effects of 61 pesticide chemicals as evaluated in experimental station and U.S. Department of Agriculture studies, on flavour, texture and other attributes such as acidity and soluble solids, pertaining to these. Summaries in English, French and German, and references quoted in the main text, conclude each chapter; and a seven-column index completes the book.

It will be clear from the above that, to begin with at least, the principal interest of "Residue Reviews" is pesticide residues; that a catholic view is taken of professional interests in these residues; and that chapters are signed by specialist authors. The book is well produced and presented, care having been taken of such difficult matters as nomenclature; common and chemical names are tabulated in three separate chapters, but an occasional inconsistency appears to have slipped through, as in the cases of TDE and DDD (pp. 21 and 61) and heptachlor. No interval, fixed or otherwise, appears to be advertised for the issue of the next volume, but this almost certainly will not be far away and will be awaited with interest by many in or otherwise directly concerned with agriculture and the food industry.

H. EGAN

ANALYTIK DES KALIUMS: KLASSISCHE UND MODERNE TRENNUNGS- UND BESTIMMUNGSMETHODEN MIT LEISTUNGS VERGLEICHEN IN KRITISCHER SICHT. By HANS TOLLERT. Pp. xvi + 416. Stuttgart: Ferdinand Enke Verlag. 1962. Price (paper) DM 75; (cloth boards) DM 79.

On the whole, this work is thorough and comprehensive, but there are a few surprising omissions. After a brief general discussion, the book is divided into three parts. The *first* (89 pages) is devoted to physical and physico-chemical techniques, flame photometry, spark and arc spectroscopy, X-ray fluorescence spectroscopy, radiometric methods, and 23 pages on all kinds of "chromatographic" separation of the alkali metals, including electrophoresis. The *second* part (130 pages) deals with chemical methods of separation and determination, every reaction that has ever been suggested seems to be included; the more important are fully treated, thus tetraphenyl boron has 27 pages, chloroplatinate 22 pages and cobaltinitrite 32 pages. The *third* part (93 pages) deals with applications, to fertiliser analysis, to the food industry, biology and medicine, water analysis, the potash industry, glass, cement, ceramics, etc.

The British analyst will find much food for thought. He will, for instance, be surprised to find that the dipicrylamine method has been developed into one of the most reliable in the potash industry. But one could wish for a firmer and more critical discussion. The author states that a solution of potassium dipicrylamine does not obey Beer's law; a few pages further on he quotes without comment a method that implies that it does. Again, Kolthoff and Bendix are quoted as saying that the reagent causes a dermatitis that is slow to heal, but the next sentence says that other authors have not reported this effect. It is of the greatest possible importance to know whether a reagent causes dermatitis or not; surely the reader is entitled to expect that an authoritative work will give him definite information on such a point. It is also rather unexpected that so much use is still made of the potassium hydrogen tartrate volumetric method, and it is the reviewer's opinion that some of the old methods described are now historical curiosities (*e.g.*, Carnot's method (1876) for precipitating potash as potassium bismuth thiosulphate, or Meyer's (1907) phosphotungstic acid method). A very brief abstract or a literature reference would suffice, and the space saved could have been devoted to more important matters that are omitted.

No British work on either the dipicrylamine method or the radioactivity method is mentioned. Far more surprising, the really important investigations of Perrin on the platinic chloride method are completely ignored. His work has been embodied in official methods for fertiliser analysis in both U.S.A. and Great Britain. The British methods are not deemed worthy of mention; it is stated that the Lindo - Gladding method is still official in U.S.A.—it was superseded 10 years ago. But the author is familiar with some of the more recent U.S.A. official methods, as he quotes the A.O.A.C. version of the volumetric tetraphenyl borate method as official. On the other hand, there is no mention that Berkhout's modification is official in Holland. Presumably the recent French official methods appeared too late for inclusion, but there is no mention at all of the use of the flame photometer in fertiliser analysis, though this has been commonplace in Great Britain and France for several years. There is, however, a good discussion of the application of flame photometry to various other materials, including milk, wine, etc. The book concludes with a list of about 1100 references and a good index.

It may be that a reviewer whose work was not concerned with fertilisers would have been more favourably impressed with the completeness of the work, which in fact does give a vast mass of information, but after all, in this country determination of potash in fertilisers is extremely important. The cost of the book is, inevitably, high. At 75 DM few individuals will buy it, but it certainly ought to be purchased by the larger chemical libraries. H. N. WILSON

BNF ANALYTICAL CONFERENCE, MALVERN, 1961. Pp. 120. London: The British Non-Ferrous Metals Research Association. 1962. Price 50s.

The Association must be congratulated on publishing, in collected form, the six papers presented at the Malvern Conference, which was opened by the Director of the B.N.F.M.R.A., G. L. Bailey; subsequently, the chairman throughout was M. Milbourn.

Large and small laboratories are faced to-day with the same vexed problem—how to handle a greater number of analyses in the least possible time, without sacrificing accuracy. It is here that this book comes into its own, because the physical and physico-chemical methods described and discussed cover a wide field, and anyone in search of instrumental methods in particular cannot fail to find some guidance in his quest. The authors are to be complimented on the lucid and authoritative manner in which their papers are presented.

The contribution by R. Lockyer, dealing with "Atomic-Absorption Spectroscopy" covers basic principles, pays particular attention to the choice of atomiser, burner, fuel, etc., and refers to the determination of copper, zinc, lead, magnesium, nickel, silver and gold in a variety of alloys, ores and concentrates.

"Application of Flame Spectrophotometry to Metallurgical Analysis," by R. A. White, is devoted to basic principles and the determination of more than 23 elements, together with their limits of detection. Methods are discussed illustrating the usefulness of this technique in the analysis of aluminium alloys, copper-base alloys, lead and lead alloys and plating solutions.

H. Hughes and G. L. Mason are the authors of the section on "Application of Optical and X-ray Spectrometric Methods to Powders and Solutions," which deals exclusively with the analysis of ferrous materials, *e.g.*, slags, iron ore and sinters; nevertheless, the underlying principles are not unrelated to the field of non-ferrous analysis; references are made to both solution and powder techniques.

"Modern Methods for the Analysis of Copper Alloys," by W. T. Elwell, makes reference to X-ray fluorescence, spectrographic, polarographic, atomic-absorption and spectrophotometric procedures. Chemical procedures include the determination of copper, lead, tin, iron, nickel, aluminium, silicon and phosphorus in complex tin-bronzes, which are embodied in B.S. 1478: Parts 1 to 8. Revised procedures for determining antimony, arsenic, bismuth and selenium are also discussed.

The paper on "Analytical Work in the Association's Chemistry Laboratories," by J. B. Price, is of more general interest; it indicates the complex nature of the various materials and solutions examined by the Association and emphasises the close co-operation between the Association's Chemistry and Physics Departments.

In "The Application of Some Modern Techniques to the Analysis of Aluminium," by R. A. Hine, several instrumental methods are discussed, and the value of the direct-reading spectrograph, in particular, is stressed. The more conventional methods of instrumental analysis are dealt with separately under their respective headings.

The sixth paper, "Physical Methods of Analysis for the Determination of Major Constituents," by K. M. Bills, deals with emission and X-ray spectrographic techniques.

An additional chapter, under the heading "Discussion," summarising the questions raised and the answers given at the Conference provides a fitting climax to the detailed information contained in this concise publication. A. CHARLESWORTH

Publications Received

COMPLEX COMPOUNDS OF TRANSURANIUM ELEMENTS. By A. D. GEL'MAN, A. I. MOSKVIN, L. M. ZAITSEV and M. P. MEFOD'EVA. Authorized translation from the Russian by C. NIGEL TURTON, B.Sc., Ph.D., and TATIANA I. TURTON, B.A. Pp. xii + 195. New York: Consultants Bureau Enterprises Inc.; London: Heywood & Company Ltd. 1962. Price 65s.

THE RADIOCHEMICAL MANUAL. Part 2. RADIOACTIVE CHEMICALS. Pp. vi + 78. Amersham, Bucks.: The Radiochemical Centre (also available from Her Majesty's Stationery Office). 1963. Price 25s.

ANALYTICAL MICROBIOLOGY. Edited by FREDERICK KAVANAGH. Pp. xvi + 707. New York and London: Academic Press Inc. 1963. Price 157s.

ORGANIC CHEMISTRY. Volume I. THE FUNDAMENTAL PRINCIPLES. By I. L. FINAR, B.Sc., Ph.D., A.R.I.C. Fourth Edition. Pp. xvi + 853. London: Longmans, Green & Co. Ltd. 1963. Price 50s.

QUIMICA DOS PESTICIDAS. By NELSON FONSECA ALMEIDA, JOSÉ RENATO PIEDADE and DÉCIO DE AGUIAR SOUZA. Pp. 325. São Paulo: Edicao do Fundo de Pesquisas do Instituto Biologico de São Paulo. 1962.

LECTURES ON GAS CHROMATOGRAPHY 1962. Edited by H. A. SZYMANSKI. Pp. vi + 282. New York: Plenum Press. 1962. Price $10.00.

Based on Lectures Presented at the Advanced Sessions of the Fourth Annual Gas Chromatography Institute held at Canisius College, Buffalo, New York, April 23rd–26th, 1962.

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Notice to Authors

LAST month we published, on pages 409 and 410, a thoroughly revised version of our "Notice to Authors," which should be read by all persons preparing papers for publication in *The Analyst.* Because of the importance of many of the changes, including some in the manuscript requirements necessitated by the simultaneous selection of two referees for every paper, the "Notice" has also been reprinted in full in the advertisement pages of this issue.

JULY, 1963 Vol. 88, No. 1048

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

SCOTTISH SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the Scottish Section with the Physical Methods Group was held on Friday, May 10th, 1963, at the Education Centre of Imperial Chemical Industries Ltd., Nobel Division, Stevenston, Ayrshire.

The subject of the meeting was "Modern Aspects of Electro-analytical Chemistry." The Chair at the morning session was taken by Dr. W. Anderson Caldwell, F.R.I.C., and the following papers were presented and discussed: "Differential Electrolytic Potentiometry; a Survey and Appraisal of Analytical Applications," by E. Bishop, B.Sc., A.R.C.S.T., F.R.I.C., R. G. Dhaneshwar, M.Sc., Ph.D., J. M. Ottaway, B.Sc., and G. D. Short, B.Sc.; "Controlled-potential Coulometric Analysis," by G. W. C. Milner, D.Sc., F.R.I.C., A.Inst. P.; "Electrochemical Approaches in Automatic Analysis," by G. Mattock, B.Sc., Ph.D., F.R.I.C. The Chair at the evening session was taken by the Chairman of the Scottish Section, Dr. R. A. Chalmers, and the following papers were presented and discussed: "Polarographic Determination of Oxygen by (*a*) the Wide-bore Dropping-mercury Electrode and (*b*) Solid Electrodes," by R. Briggs, Grad.I.E.E., and G. Knowles, B.A., B.Sc.; "Chromatopolarography and its Application to Chemical Analysis," by G. F. Reynolds, M.Sc., Ph.D., M.R.S.H., F.R.I.C. (see summaries below). The session concluded with a discussion on the use of dead-stop titrimetry in industrial analysis, which was opened by A. F. Williams, B.Sc., F.R.I.C.

During the afternoon a visit was paid to the Laboratories of Imperial Chemical Industries Ltd., Nobel Division.

DIFFERENTIAL ELECTROLYTIC POTENTIOMETRY; A SURVEY AND APPRAISAL OF ANALYTICAL APPLICATIONS

MR. E. BISHOP defined differential electrolytic potentiometry as being a method for the study of electrode processes and titrimetric reactions. It consisted in observing the potential produced across a pair of indicator electrodes immersed in the stirred titration solution by the passage of a minute heavily ballasted current. The response for reversible reactions was in the form of a first differential peak. When zero-current potentiometry gave favourable results, differential electrolytic potentiometry offered the advantages of simplicity, elimination of standard cell and salt bridge, high-speed response and enhanced precision and discrimination, but it would continue to give excellent results when zero-current potentiometry failed because of unfavourable reaction constants or low titrant concentrations. Differential electrolytic potentiometry was further uniquely advantageous on the microscale and with samples of extreme dilution; determinations of 10^{-11} mole had been satisfactorily conducted both volumetrically and coulometrically in volumes of 0·5 to 5 ml. He described the method, discussed the operational parameters and assessed its special virtues. After presenting illustrative results for all types of electron-transfer and ion-combination reactions, he gave an appraisal of the method under normal and adverse conditions and on the microscale (see, *e.g.*, *Analyst*, 1962, **87**, 845 and 860, and other parts of the same series).

CONTROLLED-POTENTIAL COULOMETRIC ANALYSIS

DR. G. W. C. MILNER discussed the principles and potentialities of this technique and described apparatus suitable for this kind of analysis. He described the advantages of this technique in unusual analytical problems in metallurgical analysis and gave a general consideration of applications in metallurgical and inorganic analysis. Finally he attempted a critical assessment of the value of controlled-potential coulometry in electro-analysis.

ELECTROCHEMICAL APPROACHES IN AUTOMATIC ANALYSIS

DR. G. MATTOCK said that automatic analysis was fundamentally more appropriate to plant process control than to laboratory control, since it bypassed the laboratory altogether in logical application. He therefore placed emphasis on automatic equipment suitable for the plant rather than the laboratory, although he recognised that there were many circumstances in which laboratory analyses were unavoidable. In the application of electrochemical methods, instruments such as automatic titrators were well known both for laboratory and plant purposes, but there were several inherently simpler measurement principles that lent themselves more readily to continuous operation, and therefore to automatic control, that had not been fully exploited. He described some recent work on electrochemical instruments, with chief reference to the sample-handling techniques necessary to render the operations automatic.

The topics discussed by the author were: direct measurements by glass electrodes, *e.g.*, for sodium, potassium and ammonium, continuous potentiometric monitoring, *e.g.*, of cyanides, simple continuous titration techniques, continuous plant coulometry and plant polarography.

POLAROGRAPHIC DETERMINATION OF OXYGEN BY (*a*) THE WIDE-BORE DROPPING-MERCURY ELECTRODE AND (*b*) SOLID ELECTRODES

MR. R. BRIGGS said that the concentration of dissolved oxygen was of paramount importance as an index of the condition of a natural water, and measurements of this constituent were necessary in almost every phase of the work of the Water Pollution Research Laboratory. The chemical methods formerly used were being superseded in many applications by physical methods. He described the recent developments in two such methods, one in which the wide-bore dropping-mercury electrode was used, and the other in which membrane-covered solid electrodes were used.

The wide-bore dropping-mercury electrode—This was based on the classical dropping-mercury electrode, but had the advantages of greater reliability, stability of calibration and greater current-carrying capacity. It consisted of an 0·8-mm capillary, waterproofed by means of a hard silicone coat, set at 45° to the horizontal and supplied with mercury through a hydraulic resistance. A silver - silver chloride reference electrode, a 1·5-volt source and a meter, complete the circuit. The electrode was kept at $-1{\cdot}5$ volts with respect to the solution, and temperature compensation was provided by means of thermistors.

A reproducibility of ± 2 per cent. over a 30° C temperature range was achieved regardless of the nature of the sample, provided a mixed reagent was added; this increased the sample conductivity, suppressed maxima and increased the pH of the sample to a value greater than 7·0.

Instruments based on this system were used extensively to indicate and record dissolved oxygen. A further application was to the measurement of the rate of respiration of the contents of "mixed liquor" tanks in which sewage in intimate contact with a biologically active floc was purified by vigorous aeration. The electrode had also been used to study liquid movements associated with polarographic maxima (G. Knowles and M. G. Keen, *J. Electroanal. Chem.*, in the press).

Solid electrodes—The dropping-mercury electrode was sensitive to turbulence in the sample, and solid electrodes were more convenient for direct measurement of dissolved oxygen in streams. Many workers had devised systems applicable to their particular problems. The most successful of these were based on the diffusion of oxygen to a polarised electrode through a permeable membrane and a film of suitable electrolyte. The theory of diffusion current for these systems had been discussed, and a solid electrode with a response time of the order of seconds described by K. H. Mancy, D. A. Okun and C. N. Reilley (*J. Electroanal. Chem.*, 1962, **4**, 65).

Recently a galvanic-cell oxygen analyser, comprising a perforated silver foil cathode (27 sq. cm in area) surrounding a lead anode formed by compressing 150 g of lead shot into the form of a cylinder, had been described by F. J. H. Mackereth (*Brit. Pat. Applic.*, 1962, 19294). The membrane consisted of polythene tubing 0·003 inch thick and the electrolyte was saturated potassium hydrogen carbonate solution. The cell had been found to have a reproducibility of $\pm 0 \cdot 5$ per cent. over periods of several months, and the current output was at least 300 μA when in equilibrium with water saturated with air at 20° C.

This comparatively large current made temperature compensation practicable, so overcoming the main disadvantage of membrane-covered electrodes, the temperature coefficient of at least 5 per cent. per ° C. By using a thermistor bridge circuit without an amplifier, compensation to within ± 1 per cent. was possible over any 10° C range, and portable indicating and recording instruments with three ranges (0° to 12° C, 10° to 22° C, 22° to 40° C) had been produced.

CHROMATOPOLAROGRAPHY AND ITS APPLICATION TO CHEMICAL ANALYSIS

DR. G. F. REYNOLDS briefly discussed the principles of chromatopolarography to illustrate that it was a technique in which both chromatography and polarography were applied to the simultaneous determination of components in a sample solution. He gave details of the apparatus and mentioned its important aspects, and also outlined the special requirements of the eluting solutions, which must act as both eluent and base electrolyte, and discussed the problems of de-oxygenation.

Dr. Reynolds described several applications to illustrate the value of the technique. He dealt in particular with its use in conjunction with reversed-phase chromatography, where the advantages of the method became apparent in the separation of mixtures of similar organic compounds whose half-wave potentials were almost coincident (see W. Kemula, *in* "Proceedings of the International Symposium on Microchemistry, Birmingham, 1958," Pergamon Press, Oxford, 1960, p. 248).

He ended by discussing some fundamental studies.

WESTERN SECTION AND ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL

A JOINT Meeting of the Western Section and the Atomic Absorption Spectroscopy Discussion Panel of the Physical Methods Group was held at 2.15 p.m. on Wednesday, April 24th, 1963, in the Department of Physical and Inorganic Chemistry, The University, Bristol. The Chair was taken by the Chairman of the Atomic Absorption Spectroscopy Discussion Panel, Mr. W. T. Elwell, F.R.I.C.

A discussion was initiated by J. A. F. Gidley, B.Sc., A.Inst.P.

MICROCHEMISTRY GROUP

THE fortieth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, May 29th, 1963, at "The Feathers," Tudor Street, London, E.C.3. The Chair was taken by the Chairman of the Group, Mr. D. W. Wilson, M.Sc., F.R.I.C.

A discussion on "Kjeldahl Nitrogen—the Digestion Process" was opened by P. R. W. Baker, M.Sc., A.R.I.C., and S. Jacobs, M.Sc., Ph.D., F.R.I.C.

The Formation of Nitrous Oxide from Hyponitrite

By J. H. ANDERSON

(*Long Ashton Research Station, Bristol*)

Solutions of sodium hyponitrite buffered at pH values from 2 to 12 produced nitrous oxide spontaneously only in the pH range 6 to 12. The similarity in range and in symmetry about the optimum (pH 9) between the curves relating pH (*i*) to the initial rate of formation of nitrous oxide from hyponitrite and (*ii*) to the calculated percentage of hydrogen hyponitrite in a hyponitrite solution suggests that nitrous oxide is formed spontaneously from hydrogen hyponitrite, but not from hyponitrous acid or the hyponitrite ion.

The amount of nitrous oxide formed accounted for the amount of sodium hyponitrite decomposed. The spontaneous formation of nitrous oxide from solutions of sodium hyponitrite buffered between pH 7 and 11 is a pseudo first-order reaction. The amount of nitrate formed from small amounts of hyponitrite by a two-stage oxidation by permanganate was approximately equivalent to hyponitrite-nitrogen. A possible mechanism for the decomposition of hydrogen hyponitrite into nitrous oxide is suggested.

THE inorganic nitrogen compound hyponitrite is reputedly unstable in aqueous neutral and acid solutions, giving rise mainly to nitrous oxide, although some workers have claimed that other inorganic nitrogen compounds are produced. It is, however, stable in alkaline solution. Frear and Burrell,[1] for instance, found that a hyponitrite solution did not decompose at pH 11·3, but at pH 7·3 it produced nitrous oxide spontaneously and rapidly without forming other nitrogen compounds. Chaudhary, Wilson and Roberts[2] suggested that hyponitrous acid decomposed to nitrous oxide as almost the sole nitrogenous product. The work described here was undertaken to investigate the rate of formation of nitrous oxide as a function of pH, and to determine the products of reaction.

Hyponitrite can be determined by oxidation to nitrite with a known excess of alkaline permanganate and subsequent oxidation of nitrite to nitrate after the mixture has been acidified.[3] This procedure was used here to study the relation between hyponitrite added and nitrate formed.

EXPERIMENTAL

PREPARATION OF HYPONITRITE—

Solid hyponitrite was prepared as the sodium salt by Addison, Gamlen and Thompson's method[4] and as the silver salt by Medina and Nicholas's method.[5] These preparations were not contaminated by nitrite, nitrate or ammonia; they were free from hydroxylamine, giving no reaction when tested as described by Frear and Burrell,[1] and only small amounts of azo-dye after iodine oxidation by a modification of Czsaky's method.[6] The sodium hyponitrite preparation contained carbonate.

A solution of sodium hyponitrite was prepared from the silver salt by adding the solid to a small excess of 0·5 N hydrochloric acid. After the precipitate of silver chloride had been removed by centrifugation, a sample of the clear supernatant liquid was rapidly mixed with an excess of sodium hydroxide solution to make the sodium hydroxide concentration 0·2 N. This solution, and a solution prepared by dissolving solid sodium hyponitrite in 0·2 N sodium hydroxide, had a light absorption peak at 248 mμ, which is in agreement with previous findings for hyponitrite.[4] Solutions of solid sodium hyponitrite were used for the experiments described below. Reagents were of analytical grade and de-ionised water was used.

FORMATION OF NITROUS OXIDE—

The formation of nitrous oxide by decomposition of hyponitrite was followed manometrically at 30° C in conventional Warburg constant-volume respirometers[7] and 16-ml flasks. In the experiment represented in curve A, Fig. 1 (see p. 498), flasks contained 2·3 ml of buffer solution of known pH in the range 2 to 12 (prepared with use of a wide-range glass electrode) and 0·2 ml of 2 N potassium hydroxide *plus* filter-paper in the centre well. Buffer solutions of pH 2 to 7·9 contained 0·1 M pyrophosphate and 0·1 M citrate; buffer solutions of pH 8 to 12 contained 0·1 M pyrophosphate, 0·1 M borate and 0·1 M carbonate.

After temperature equilibration, 0·2 ml of a solution of sodium hyponitrite (43·2 mg of solid sodium hyponitrite dissolved in 8 ml of 0·1 N sodium hydroxide) was tipped in, and flasks were shaken at 80 cycles per minute. The pH values of the reactions were measured after the experiments; these are the values shown on curve A, Fig. 1. The increase in pH caused by the formation of hydroxyl ions from hyponitrite was not greater than 0·3 pH unit. The initial rate of a reaction was calculated from its reaction velocity curve at the point where the formation of nitrous oxide was 15 per cent. of the total evolved.

A similar technique was used in the nitrogen balance experiments recorded in Table I, except that the reaction was begun by adding 8·1 mg of solid sodium hyponitrite through the side-arm of a flask to 3 ml of M phosphate buffer solution or 0·1 N sodium hydroxide in the main compartment.

TABLE I

RELATION BETWEEN HYPONITRITE ADDED, NITROUS OXIDE FORMATION AT VARIOUS pH VALUES AND RESIDUAL HYPONITRITE

pH of buffer	N_2O formed, μmoles	Rate of N_2O formation, μmoles per 20 minutes	Weight of solid $Na_2N_2O_2$ added, mg	Residual nitrate, μmoles	Residual hyponitrite determined in samples, μmoles	Total inorganic nitrogen, μmoles
1	32·1	8	8·1	0·75	7·7	40·2
3	8·3	1·6	8·0	0·6	22·7	31·3
5	22·4	3·2	8·7	0	12·0	34·4
7	39·2	18	8·1	0	0	39·2
9	38·1	23	8·1	0	0	38·1
11	40·1	7	8·1	0	0	40·1
(0·1 N NaOH)	18·3	3	8·1	0	21·6	39·9

Carbonate in solid sodium hyponitrite was measured manometrically by differential absorption (by potassium hydroxide) of carbon dioxide formed when a solution of the hyponitrite was added to 0·5 N sulphuric acid.

DETERMINATION OF INORGANIC NITROGEN COMPOUNDS—

(*a*) Ammonia in samples was determined as described by Conway.[8]

(*b*) Nitrite was determined colorimetrically by the modification of Nicholas and Nason's[9] method described below. Samples containing 0·005 to 0·200 μmole of nitrite were diluted to 7 ml with water, and 1 ml of sulphanilamide reagent (1 per cent. of sulphanilamide in 2 N sulphuric acid) was added. After 2 minutes, 2 ml of a 0·02 per cent. aqueous solution of *N*-1-naphthylethylenediamine dihydrochloride were added. The light absorption of the resulting azo-dye was determined after 10 minutes in an E.E.L. (Evans Electroselenium Ltd.) colorimeter with a green filter (OGRI; maximum transmission at 530 mμ), against a reagent blank solution. The amount of nitrite in the sample was found from a calibration curve for solutions of sodium nitrite treated in the same way.

(*c*) Nitrate in the presence of nitrite was determined by a modification of Middleton's method[10] for quantitatively reducing nitrate to nitrite. A solution containing 0·25 to 5 μmoles of nitrate *plus* nitrite was diluted with water to 7·5 ml in a round-bottomed glass tube (diameter 2·3 cm; length 10·5 cm). The addition of 2·5 ml of 4 N ammonium hydroxide brought the pH of the solution to 11·6 and the volume to 10 ml. Nitrite present was determined in a 0·25- to 1·0-ml sample diluted to 7 ml with water. The reduction of nitrate to nitrite was initiated by the addition of 100 mg of zinc powder, maintained in suspension by shaking the reaction mixture at 300 cycles per minute on a reciprocal shaker. The reaction was stopped after 5 minutes by spinning in a centrifuge at 500 **g** for 1 minute. Nitrite was determined in 0·25 to 1 ml of the clear supernatant liquid. Nitrite was also measured in the supernatant liquid of a reagent blank solution, in which nitrite and nitrate solutions had been replaced by water. This value, which accounted for traces of nitrite or nitrate present in the zinc powder, was always deducted from the value of nitrite determined.

Before use, the method was examined:

1. The recovery of 0·25 to 5 μmoles of sodium nitrite (without added nitrate) was 90 to 96 per cent.; mean recovery 92 per cent.

2. The recovery of nitrite formed from 0·25 to 5·0 μmoles of potassium nitrate (without added nitrite) was 89 to 96 per cent. of theoretical; mean recovery 91 per cent.

The similarity between the mean recoveries shows that the reduction of nitrate to nitrite is 99 per cent. complete. The mean recoveries were not affected by the presence of 25 μmoles of manganous sulphate. Manganous ions, although used by Middleton,[10] therefore appear to be unnecessary. The recovery of added nitrite was not affected by phosphate or pyrophosphate, but, because these compounds diminished the rate of reduction of nitrate to nitrite, it was necessary to re-determine the recovery of nitrite formed from added nitrate. Experimentally determined values for nitrate were found by multiplying the recovery of nitrite by the appropriate factors derived from these recovery experiments. Before the nitrate in samples of reaction mixtures was determined it was necessary to adjust them to pH 8 by adding hydrochloric acid or sodium hydroxide, 0·05 ml of 0·005 M phenolphthalein in 70 per cent. ethanol being used as indicator. The subsequent addition of ammonium hydroxide brought the solution within the pH range (11 to 11·6) required for the reduction of nitrate to nitrite.

(*d*) Hyponitrite was determined by a two-stage oxidation with excess of permanganate[3] and subsequent determination of the nitrate formed. One millilitre of hyponitrite was mixed with 0·5 ml of 2 N sodium hydroxide in a round-bottomed tube, as used for the nitrate determination; 0·25 ml of 0·1 M potassium permanganate was then added, and the oxidation of hyponitrite to nitrite was allowed to proceed for 7 minutes (stage 1). The addition of 0·5 ml of a solution of 0·005 M manganous sulphate in 6 N sulphuric acid acidified the permanganate, which was then allowed to oxidise nitrite to nitrate for 7 minutes (stage 2). A pre-determined volume (approximately 0·5 ml) of 0·2 M sodium oxalate, sufficient to reduce all the permanganate to colourless manganous ions in 5 minutes, was added. After a further 2 minutes, 0·05 ml of phenolphthalein indicator was added down the side of the tube, and the mixture was adjusted to pH 8 by carefully adding 2 N sodium hydroxide solution with swirling. There was no nitrite present in this mixture. The mixture was diluted to 7·5 ml with water, and then 2·5 ml of 4 N ammonium hydroxide were added, which brought the pH to 11·0 to 11·6. Zinc powder (100 mg) was then added, and the mixture was shaken for 5 minutes, as described in the nitrate determination. After the zinc powder and the precipitate of manganese hydroxide had been removed by centrifugation, nitrite was determined in samples of the clear supernatant liquid. Values were always corrected by subtracting nitrite found in a reagent blank solution, in which water replaced the hyponitrite solution. When solutions containing 0·5 to 5 μmoles of nitrite or nitrate were treated in this way, the recovery of added nitrite was 78 to 87 per cent. of theoretical (mean recovery 85 per cent.) and the recovery of nitrite formed from added nitrate was 75 to 86 per cent. of theoretical (mean recovery 83 per cent.). The similarity of the two mean recoveries suggests that nitrite is quantitatively oxidised to nitrate by acid permanganate. The observation that no nitrite was present in determinations before reduction with zinc and ammonia is consistent with this suggestion.

The oxidation of hyponitrite by alkaline permanganate produces nitrite,[3] but, since it is possible that nitrate may be simultaneously produced, the determination must be regarded essentially as a method of determining nitrate. When nitrate and nitrite were present with hyponitrite, their concentrations were subtracted from the amount of nitrate formed from hyponitrite (*e.g.*, see Table I).

A fixed amount (2 μmoles) of nitrite was used in replicate determinations, and the recoveries were within the range 78 to 87 per cent. when the periods of reaction were varied independently within the time limits: oxidation with alkaline permanganate (stage 1), 5 to 15 minutes; oxidation with acid permanganate (stage 2), 2 to 10 minutes; reduction by oxalate (total period), 7 to 10 minutes. These results show that in the determination there is no significant conversion of nitrate or nitrite to nitrogenous gases, which could disappear from solution.

When the permanganate was reduced by acidified solutions of ferrous sulphate or sodium arsenite the recoveries of nitrite were low. Probably these reagents in acid conditions reduced nitrate to nitrogenous gases (nitrogen or nitrous oxide). It was important to minimise the amount of oxalate added, because a large excess diminished the rate of reduction of nitrate to nitrite.

STOICHEIOMETRY OF HYPONITRITE OXIDATION—

In the determination of hyponitrite, the crucial stage is the oxidation by alkaline permanganate, because nitrous oxide may also be produced. Stage 1 was studied manometrically to measure nitrous oxide formed. The amount of hyponitrite oxidised was found from the equation—

$$N_2O_2^{2-} = 2NO_3^-$$

after the hyponitrite determination had been completed. The amounts of nitrate and nitrous oxide formed were then compared with the amounts of hyponitrite added, which was measured as nitrous oxide produced when a similar sample completely decomposed at approximately pH 8.

When 3·2 μmoles of hyponitrite in 0·2 ml of 0·2 N sodium hydroxide were added to a solution of 1 ml of 0·1 M potassium permanganate *plus* 1·3 ml of 0·16 N sodium hydroxide in a Warburg flask, 0·1 μmole of nitrous oxide was immediately formed, and 5·7 μmoles of nitrate were formed from a sample removed after 10 minutes. Thus, of the 3·2 μmoles of hyponitrite added, 89 per cent. was oxidised, 3 per cent. was converted to nitrous oxide and 8 per cent. could not be accounted for. Nitrous oxide formation is therefore not significant during alkaline oxidation.

When, however, the hyponitrite solution was added to a solution of 1·3 ml of 0·16 N sulphuric acid *plus* 1 ml of 0·1 M permanganate, 78 per cent. appeared as nitrous oxide, 16 per cent. was oxidised to nitrate and 6 per cent. could not be accounted for. It seems that permanganate catalyses the formation of nitrous oxide from hyponitrous acid in sulphuric acid. Similar results were obtained when sodium hyponitrite solutions prepared from silver hyponitrite were mixed with alkaline or acid permanganate.

When hydroxylamine sulphate was added to alkaline or acid permanganate, nitrate and large amounts of nitrogenous gases were formed; hydrazine sulphate formed nitrogenous gases, but no nitrate.

RESULTS

Table I shows results of nitrogen-balance experiments in which the amount of hyponitrite added was related to the amount of nitrous oxide formed at various pH values and the residual amount of hyponitrite. The column, "total inorganic nitrogen," gives the sum of nitrous oxide formed, the residual hyponitrite and the amount of hyponitrite converted to nitrate in the manometric reaction. Since the purpose of the experiments was to make a nitrogen balance, the duration of a reaction, in the range 2 to 6 hours, was not critical. During this period, *e.g.*, after 2 hours in the experiment at pH 9, a sample was removed, mixed with sodium hydroxide, and the determination of hyponitrite was begun immediately. No nitrite, nitrate, hydroxylamine or ammonia was found in further samples. The small amount of nitrate formed in reactions at pH 1 and 3 was typical of that occasionally produced at low pH values.

At pH 7, 9 and 11 the hyponitrite rapidly and completely decomposed. The amount of nitrous oxide formed (mean, 39·1 μmoles) thus accounts for the hyponitrite disappearing. The sum of nitrous oxide formed and residual hyponitrite in 0·1 N sodium hydroxide is 39·9 μmoles. According to the equation—

$$Na_2N_2O_2 + H_2O = 2NaOH + N_2O$$

39·1 μmoles correspond to 4·15 mg of sodium hyponitrite or 51 per cent. of the weight used. Carbonate (Na_2CO_3) accounts for 36 per cent.; water might account for the remaining 13 per cent.

The relation between the pH of the reaction and initial rate of formation of nitrous oxide is shown in Fig. 1 (curve A). Formation of nitrous oxide occurs in the pH range 6 to 12 with a symmetrical optimum at pH 9. In similar experiments lasting 90 minutes, in which hyponitrite solutions were added to 0·5 N sulphuric acid or 0·2 N sodium hydroxide, there was negligible formation of nitrous oxide.

The amounts of nitrous oxide formed when reactions were allowed to proceed to completion (approximately 90 minutes), were the same throughout the pH range 7 to 11 (mean value 9·1 μmoles ± 4 per cent.). No hyponitrite, nitrate, hydroxylamine or ammonia and only about 0·005 μmole of nitrite was found in the residual reaction mixtures. In reactions at pH values outside this range, similar amounts of nitrite, but no nitrate, hydroxylamine or ammonia, were found. The mean value of nitrous oxide was obtained from 0·2 ml of hyponitrite solution (43·2 mg per 8 ml). This solid preparation thus contained about 90 per cent. of sodium hyponitrite. Results similar to those in Fig. 1 (curve A) were obtained with a solution of sodium hyponitrite prepared from silver hyponitrite.

The relation between the rate of formation of nitrous oxide and pH in Table I bears a general resemblance to Fig. 1 (curve A). The finding (see Table I) that nitrous oxide was formed rapidly at pH 1 contrasted with the general observation throughout this work (see Fig. 1, curve A) that hyponitrite was stable in acid solutions. Possibly some impurity in the reagents catalysed this reaction and the formation of small amounts of nitrate (pH 1 and pH 3 in Table I). At pH 3 and pH 5 the sum of the nitrous oxide appearing and the residual hyponitrite is less than the expected amount of 39·1 μmoles. It is likely that at these pH values some formation of nitrous oxide occurred as the acid reaction mixtures added to sodium hydroxide (stage 1) passed transiently through a pH range of 6 to 12.

Fig. 2 shows the relation between the logarithm of the hyponitrite concentration remaining at time *t* (minutes) after the initiation of a manometric experiment at pH 8·6 and *t*. The amount of hyponitrite present at time *t* was found by subtracting the manometric reading at time *t* from the stationary reading observed at the end of the experiment. The linear relationship shows that the spontaneous decomposition of hyponitrite is a pseudo first-order reaction. Similar straight lines were obtained at pH values from 7 to 11.

Discussion of the work

Chaudhary, Wilson and Roberts[2] found that, at pH 7·3, hyponitrite disappears according to a first-order reaction. Frear and Burrell[1] found that hyponitrite is stable at pH 11·3, but at pH 7·3 it decomposes with a half-life of 11 minutes, forming only nitrous oxide. The finding (Fig. 1, curve A) that formation of nitrous oxide occurs spontaneously in the pH range 6 to 12 is in agreement with these results. Since the reactions in the pH range from 7 to 11 are complete with the formation of no nitrate, hydroxylamine or ammonia and only traces of nitrite (Fig. 1, curve A), the amount of nitrous oxide spontaneously formed may be used as a measure of the decomposition of hyponitrite. In residual reaction mixtures outside this pH range no formation of nitrogen compounds other than nitrous oxide was found. Formations of nitrous oxide from hyponitrite in acid conditions were occasionally observed (*e.g.*, pH 1 of Table I). These reactions and formation of nitrate may be catalysed by some impurity in the reagents.

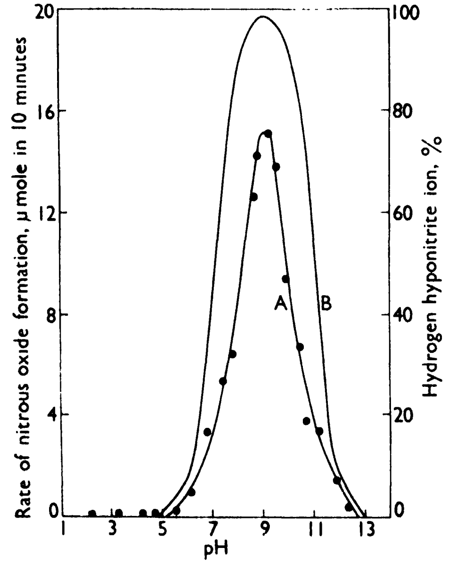

Fig. 1. The relation between pH and: curve A, the initial rate of formation of nitrous oxide from hyponitrite; curve B, the calculated percentage of hydrogen hyponitrite

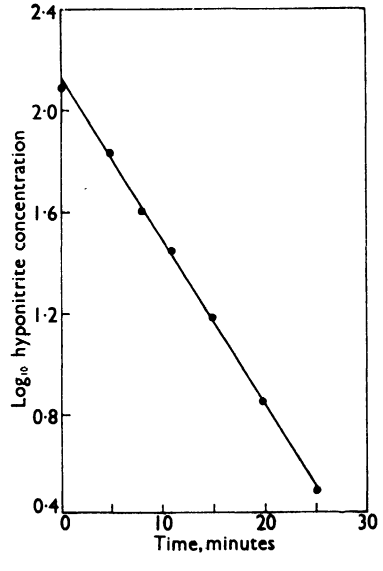

Fig. 2. The relation between $\log_{10}$ hyponitrite concentration remaining at time *t* and *t*. Reaction mixture: volume 2·5 ml; pH 8·6; 7·8 μmoles of hyponitrite. The rate constant was 0·152 minutes^{-1} for a first-order reaction

Latimer[11] gives the values below for the acid dissociation constants of hyponitrous acid—

$$H_2N_2O_2 = HN_2O_2^- + H^+;\ K_1 = 9 \times 10^{-8}$$
$$HN_2O_2^- = N_2O_2^{2-} + H^+;\ K_2 = 1 \times 10^{-11}$$

The percentage of hydrogen hyponitrite ion in a hyponitrite solution at a given hydrogen ion concentration can be calculated from the equation—

$$\frac{[HA]}{[A_T]} = \frac{100}{\frac{[H]}{K_1} + \frac{K_2}{[H]} + 1}$$

where $[A]$ = concentration of $N_2O_2^{2-}$,
$[HA]$ = concentration of $HN_2O_2^-$,
$[H_2A]$ = concentration of $H_2N_2O_2$,
$[A_T]$ = $[A] + [HA] + [H_2A]$ and
$[H]$ = concentration of hydrogen ions.

The relation between the pH of a solution of hyponitrite and the percentage of hydrogen hyponitrite ion calculated in this way (see Fig. 1, curve B) is a symmetrical curve (pH 5 to 13) with a peak at pH 9. The similarity between the observed results (Fig. 1, curve A) and the theoretical findings (Fig. 1, curve B) suggests that the initial rate of nitrous oxide formation from hyponitrite is a function of the hydrogen hyponitrite ion concentration and that hyponitrous acid and the hyponitrite ion are stable. The equation below would thus describe the spontaneous decomposition of hyponitrite—

$$HN_2O_2^- \rightarrow N_2O + OH^-.$$

The spontaneous formation of nitrous oxide from hyponitrite at pH values in the range 7 to 11 (*e.g.*, pH 8·6, Fig. 2) is a pseudo first-order reaction. This is in agreement with previous findings.[1,2] The molecule of hydrogen hyponitrite, $HN_2O_2^-$, contains one hydroxyl group with the hydrogen dissociated and one with the hydrogen not dissociated. Possibly the spontaneous decomposition of hydrogen hyponitrite proceeds by an intra- or inter-molecular mechanism involving bonding between O^- and OH.

The oxidation of hyponitrite to nitrite by alkaline permanganate produces so little nitrous oxide that the nitrate subsequently formed from nitrite by acidifying the permanganate is approximately equivalent to the hyponitrite-nitrogen added (*e.g.*, see Table I). In using alkaline permanganate for determining hyponitrite, it is obviously necessary to take the precaution of measuring any nitrogenous gases produced.

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Precipitation of Cuprous Thiocyanate from Homogeneous Solution

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A method for precipitating cuprous thiocyanate from homogeneous solution is described, in which the character of the precipitate is governed by the rate of the reduction of cupric ions to the cuprous state. The method is simple and accurate; its main advantages over conventional methods are an over-all reduction in the time required for the determination, and the production of a precipitate that is easy to manipulate and to collect by filtration.

THE insolubility of cuprous thiocyanate and the relatively high solubilities of the thiocyanates of many other metals provides a selective method for quantitatively separating copper from a solution containing other metals, as well as providing a gravimetric method for its determination. Consequently, since its introduction by Rivot in 1854,[1] the quantitative precipitation of cuprous thiocyanate has been widely applied.

Rivot[1] precipitated cuprous thiocyanate from hydrochloric acid solution with potassium thiocyanate, after reduction of cupric copper to the cuprous state with sulphite. He applied the method to the determination of copper in bronzes, but gave few practical details.

This method was used by van Name[2,3,4] for determining copper in coinage metals. He reported that only a small amount of free acid should be present during precipitation and that the precipitate should be set aside for 20 hours before filtration. Precipitation of tin, arsenic, antimony and bismuth was prevented by adding tartaric acid.

Kolthoff and van der Meene[5] studied in detail the conditions for precipitating cuprous thiocyanate. They concluded that the concentration of free acid should not exceed 0·5 N and that the concentration of the excess of thiocyanate precipitant should be less than 0·05 N. Reduction of cupric ions with sulphite was best performed in boiling solution.

Similar conclusions on the maximum permissible concentrations of acid and excess of precipitant were reported by Belcher and West,[6] who used ammonium ferrous sulphate as the reductant in the cuprous thiocyanate method.

Most workers consider it necessary to age the precipitated cuprous thiocyanate before filtration, although there are disagreements about the recommended time periods. Thus 2,[7] 6,[6,8] 20,[2,3,4] 24[5] and "several"[9] hours have been specified.

Stathis[10] used ascorbic acid to reduce cupric ions in the thiocyanate method. Whereas previous workers[2,5] had found that oxidising agents must be excluded, Stathis obtained quantitative precipitation of cuprous thiocyanate from nitric acid solutions of copper alloys. Moreover, the time period between precipitation and filtration was reduced to 30 minutes.

EXPERIMENTAL

The main disadvantage of the cuprous thiocyanate method is that the rather fine precipitate generally produced may clog or even pass through the filter. It has a marked tendency to "creep" with consequent risk of loss in transferring it to the filter and difficulty during washing. Heating the solution, even to boiling, with constant stirring, improves the granularity of the wetted precipitate, but is only partly effective in dealing with the precipitate that has crept up the walls of the container.

The work described here was undertaken to produce cuprous thiocyanate with good filtration characteristics and that does not require ageing before filtration.

The technique of precipitation from homogeneous solution[11] depends on the production of one of the reactants in the presence of the others at a rate such that a controlled growth of the crystals of the insoluble product results.

To achieve homogeneous precipitation of cuprous thiocyanate, it was decided to control the rate of production of cuprous ions. Three methods of approach can be envisaged for achieving this, namely, (1) the slow liberation of cupric ions from a complex compound into a reducing medium, (2) the generation of a reducing agent in a solution containing cupric ions and (3) the production of conditions under which the reaction of cupric ions with a reducing agent is delayed.

Successful precipitation of cuprous thiocyanate from homogeneous solution was achieved by using each of these three approaches. Thus, it was possible to precipitate cuprous thiocyanate from homogeneous solutions containing amminocupric ions. Ammonia solution was added to cupric sulphate solution until the cupric hydroxide precipitate redissolved. The solution remained clear when ammonium thiocyanate and sodium sulphite were added, but when the solution was heated ammonia was expelled and cuprous thiocyanate was precipitated. This procedure would, however, have no practical value for determining copper in the presence of metals having insoluble hydroxides.

The controlled generation of a reducing agent was obtained by the hydrolysis of sucrose. Sucrose was added to slightly acidic solutions containing cupric and thiocyanate ions. The solutions remained clear at room temperature, but when they were heated precipitation of cuprous thiocyanate began after a few minutes. The cupric ions were reduced by the glucose and fructose produced by hydrolysis of the sucrose. In this way highly crystalline cuprous thiocyanate with excellent filtration characteristics was produced. Unfortunately, it was impossible to precipitate the copper quantitatively in less than 4 hours, and strict control of pH was necessary. Solutions having acidities in excess of 0·03 N in hydrochloric or sulphuric acid gave darkened precipitates, and the supernatant liquid developed an opalescence and a sulphurous odour. This was attributed to further reaction of cuprous thiocyanate with fructose to produce cuprous sulphide. Nevertheless, with proper control of the conditions, successful gravimetric determinations of copper were made, but this method was abandoned because of practical difficulties.

Delayed reduction of the cupric ion was reported by Davis[12] in the homogeneous precipitation of cuprous tetraphenylborate. Ascorbic acid was added to acid solutions containing cupric and tetraphenylborate ions, and precipitation did not occur until about half a minute had elapsed. This method was not applicable to cuprous thiocyanate; immediate precipitation occurred under a wide range of temperature and acidity conditions when ascorbic acid was added to solutions containing cupric and thiocyanate ions.

With hydroxyammonium chloride, which is a milder reducing agent than ascorbic acid, delayed reduction of cupric ions was achieved by temperature control. A solution of hydroxyammonium chloride was added to a solution of cupric and thiocyanate ions, with an acidity of about 0·2 N, without precipitation occurring. When this solution was heated on a steam-bath, precipitation of cuprous thiocyanate began within a few minutes and appeared to be complete within about half an hour. The cuprous thiocyanate produced in this way was granular and had excellent filtration properties. A study of the conditions for precipitation resulted in the development of an accurate method for the gravimetric determination of copper.

INVESTIGATION OF PRECIPITATION CONDITIONS—

An investigation of the reaction variables was carried out to find conditions under which precipitation of copper was complete within the limits of good gravimetric accuracy. These conditions were then applied to gravimetric determinations on solutions containing known amounts of copper, to check the procedure.

Preliminary experiments were performed in which completeness of precipitation was judged visually. When ammonium or potassium thiocyanate is added to a solution of a cupric salt, a green colour is produced, presumably due to the formation of a complex between cupric and thiocyanate ions. The disappearance of this colour when the complex is treated with hydroxyammonium chloride and heated is a good indication that precipitation is complete. These experiments led to the adoption of the reagents used in the subsequent tests. It was found expedient to use a colorimetric micro-titration procedure for determining the amount of copper remaining in the filtrate after precipitation of the cuprous thiocyanate.

Portions (50 ml) of a 1 per cent. solution of cupric sulphate were transferred to separate 150-ml Phillips beakers. To each solution were added in the stated order the amounts of hydrochloric acid, sp.gr. 1·18, 3·5 per cent. ammonium thiocyanate solution and 3 per cent. hydroxyammonium chloride solution specified in Table I, the solutions being stirred after each addition. The solutions were heated on a steam-bath for 30 minutes, cooled to room temperature and set aside for 10 minutes.

After filtration through No. 4 porosity sintered-glass crucibles, 5 ml of each filtrate were transferred to a separating funnel containing 8 ml of water and 2 ml of 5 N sulphuric acid, and treated as described below.

Five millilitres of a 0·1 per cent. solution of diethylammonium diethyldithiocarbamate were added, and the mixture was shaken vigorously for 2 minutes and allowed to separate. Any copper remaining in the 5-ml portion of filtrate was extracted into the organic layer as the intense yellow copper dithiocarbamate. A blank solution, containing 50 ml of water in place of the cupric sulphate solution, was prepared, and, with each test, 5 ml were taken through the procedure described above.

The blank solution was titrated from a 2-ml burette with dilute standard cupric sulphate solution (1 ml ≡ 10 µg of copper), the mixture being shaken vigorously after each increment and allowed to separate. This was continued until the colour of the organic layer of the blank matched that of the test. The volume of titrant required was therefore a measure of the amount of copper in 5 ml of filtrate.

RESULTS—

Results obtained in this way are shown in Table I. The last column shows the calculated negative error due to the amount of copper remaining in the whole filtrate.

TABLE I

EFFECTS OF DIFFERENT CONCENTRATIONS OF THE REAGENTS

Hydrochloric acid added, ml	Ammonium thiocyanate solution added, ml	Hydroxyammonium chloride solution added, ml	Copper in filtrate, %
1·0	7·5	15	0·17
1·0	10	15	0·06
1·0	12·5	15	0·05
1·0	15	15	0·03
1·0	20	15	0·05
1·0	15	5	0·13
1·0	15	10	0·04
1·0	15	15	0·03
1·0	15	20	0·03
0·5	15	15	0·03
1·0	15	15	0·03
1·5	15	15	0·03
1·8	15	15	0·09
2·0	15	15	0·25

DISCUSSION OF RESULTS—

The optimum conditions derived from the results shown in Table I require 15 ml each of ammonium thiocyanate solution and hydroxyammonium chloride solution, and about 1 ml of hydrochloric acid. The concentration of acid should not exceed 0·3 N, and the concentration of the excess of ammonium thiocyanate should not exceed 0·07 M. Similar results were obtained by using a 4·5 per cent. solution of potassium thiocyanate (≡ 3·5 per cent. ammonium thiocyanate) and with sulphuric or perchloric acid instead of hydrochloric acid. It was also found that nitric acid in concentrations less than 0·1 N did not affect the results.

When the optimum reagent concentrations were used it was found that 20 minutes' heating was insufficient (error 0·33 per cent.), but that heating for longer than 30 minutes was unnecessary.

METHOD FOR THE GRAVIMETRIC DETERMINATION OF COPPER

The precipitation conditions derived were applied to the gravimetric determination of copper in solutions prepared from pure electrolytic copper foil and copper-bearing alloys. After the cuprous thiocyanate had been precipitated and collected, it was washed first with a dilute solution of ammonium thiocyanate containing a little ascorbic acid (to maintain reducing conditions) and finally with dilute ethanol to remove any excess of ammonium thiocyanate.

PROCEDURE—

Transfer 50 ml of acidified copper solution, containing about 125 mg of copper and having an acid concentration of less than 0·3 N, to a clean unscratched 150-ml Phillips beaker. Add, with mixing, 15 ml of ammonium thiocyanate solution and 15 ml of hydroxyammonium chloride solution. Heat the solution on a steam-bath for 30 minutes, cool it to room temperature, and set aside for 10 minutes. Collect the precipitate on a previously dried and weighed No. 4 porosity sintered-glass crucible, and use the wash solution to complete the transfer of the precipitate. Wash the precipitate a few times with wash solution and finally with dilute ethanol. Dry the crucible and its contents for 2 hours at 110° C, set aside to cool, and weigh.

1 g of cuprous thiocyanate = 0·5226 g of copper.

APPLICATION OF THE METHOD TO COPPER METAL

Pure electrolytic copper foil (2·4395 g), freshly de-greased and washed with dilute nitric acid, was dissolved in 3 ml of nitric acid, sp.gr. 1·42, and 3 ml of water. When dissolution was complete, 5 ml of sulphuric acid, sp.gr. 1·84, were added, and the mixture was evaporated to small volume to expel nitric acid. When it was cool, the mixture was diluted with 20 ml of water and again evaporated to small volume; this treatment was repeated twice. The solution was then transferred with water to a 1-litre calibrated flask and diluted to the mark with water at 20° C.

This solution was about 0·18 N in sulphuric acid, and 50 ml contained 0·1220 g of copper. Twelve 50·0-ml portions were tested by the method described above, and the weights of copper calculated from the weights of cuprous thiocyanate obtained lay within the range 0·1219 to 0·1221 g with a mean value of 0·1220 g. By assuming the copper to be 100·0 per cent. pure, the recoveries were thus 100·0 per cent. to within ±0·08 per cent. The standard deviation was 0·067 per cent.

APPLICATION OF THE METHOD TO COPPER ALLOYS

Four British Chemical Standard alloys of copper were analysed by this method. Two solutions, one in sulphuric acid and the other in perchloric acid, were prepared from two of the alloys and one solution was prepared from each of the other alloys.

Preparation of Alloy Solutions

CUPRO-NICKEL 180/1—

In sulphuric acid—A 0·9299-g sample of the alloy was placed in a 150-ml Kjeldahl flask, and 3 ml each of water and nitric acid, sp.gr. 1·42, were added. After the metal had dissolved, 1·5 ml of sulphuric acid, sp.gr. 1·84, were added, and the solution was evaporated to small volume to expel the nitric acid. The solution was then cooled, diluted with 50 ml of water, and neutralised to pH 4 with ammonia solution, sp.gr. 0·89. It was then transferred with water to a 250-ml calibrated flask, 15 ml of 5 N sulphuric acid were added, and the solution was made up to the mark at 20° C with water.

In perchloric acid—A 0·9301-g sample of the alloy was weighed into a tall 150-ml beaker and heated with 7·5 ml of perchloric acid, sp.gr. 1·54, until it dissolved. When cool, the solution was transferred with water to a 250-ml calibrated flask, and the solution was diluted to the mark with water.

MANGANESE-BRASS "B" 179—

In sulphuric acid—A 1·0601-g sample of the alloy was weighed into a 150-ml Kjeldahl flask, 4 ml each of water and nitric acid were added, and occasional heating was applied until dissolution was complete. Two millilitres of sulphuric acid were added, and the solution was heated to fumes for 5 minutes. When cool, the solution was diluted with about 20 ml of water, and again heated to fumes for 5 minutes. It was then cooled and diluted with 50 ml of water. The turbid solution produced was filtered through a Whatman No. 42 filter-paper into a 250-ml flask, and the filter was washed with water. The filtrate and washings were diluted to the mark with water.

In perchloric acid—A 1·0727-g sample was transferred to a tall 150-ml beaker and dissolved, with warming, in 9·5 ml of perchloric acid. When cool, the solution was diluted with 50 ml of water and filtered through paper into a 250-ml calibrated flask. The filter was washed with water, and the filtrate and washings were diluted to the mark with water.

Bronze 183/1—

A 0·7301-g sample was dissolved in a mixture of 8 ml of water and 12 ml of nitric acid contained in a 150-ml beaker. The solution was evaporated by heating on a steam-bath to reduce the volume to between 5 and 10 ml. It was then diluted with 50 ml of hot water, and digested on the steam-bath for a further 15 minutes. The precipitate of metastannic acid was removed by filtration through a No. 42 filter-paper, and the filter was washed 10 times with a few millilitres of dilute nitric acid (1 + 20). The filtrate and washings were evaporated to small volume (but not to dryness); the concentrated solution was then diluted with 50 ml of water, and neutralised to pH 4 with ammonia solution. The solution was transferred to a 250-ml calibrated flask, acidified with 5 ml of perchloric acid, and diluted to the mark with water.

Bronze "C" 207—

A 0·7302-g sample was heated in a tall 150-ml beaker with 9 ml of perchloric acid and 1·5 ml of nitric acid. The solution was then heated to fumes until colourless, diluted with 50 ml of hot water, and digested for 15 minutes on a steam-bath. It was then filtered through No. 42 filter-paper (to remove the metastannic acid precipitate) into a 250-ml calibrated flask. The filter was washed with 100 ml of water containing 1 ml of perchloric acid, and the filtrate and washings were diluted to the mark with water.

Procedure

Copper was determined in three 50-ml portions of each solution by the method described, except that 0·5 g of sodium hydrogen tartrate was added to each portion of the solution prepared from Bronze 183/1 before the other reagents were added. This alloy contained 0·24 per cent. of antimony and 0·14 per cent. of arsenic, both of which produce precipitates under the conditions of the test unless tartrate is added to complex them. The results obtained are shown in Table II and are compared with the certified copper contents.

TABLE II

RESULTS OBTAINED WITH COPPER ALLOYS

Alloy	B.C.S. reference number	Method of preparing solution	Copper found, %	Mean copper found, %	Certified copper value, %	Range of copper values certified, %
Cupro-nickel	180/1	In H_2SO_4	67·35	67·34	67·36	67·32 to 67·40
			67·35			
			67·32			
		In $HClO_4$	67·33	67·33		
			67·30			
			67·36			
Manganese brass "B"	179	In H_2SO_4	58·73	58·73	58·8	58·6 to 59·06
			58·71			
			58·75			
		In $HClO_4$	58·70	58·70		
			58·73			
			58·67			
Bronze	183/1	—	84·68	84·71	84·8	84·75 to 84·95
			84·68			
			84·76			
Bronze "C"	207	—	86·76	86·79	86·84	86·65 to 87·00
			86·76			
			86·84			

RESULTS

All the results obtained are in good agreement with the certified copper contents, and the replicate determinations on each alloy agree well. The other constituents of the alloys do not, therefore, interfere in the determination. The certified amounts of the other metals are shown in Table III.

TABLE III

OTHER CONSTITUENTS OF THE ALLOYS

Alloy	Constituents other than copper
Cupro-nickel 180/1	30·85 per cent. of Ni; 0·82 per cent. of Fe; 0·81 per cent. of Mn
Manganese brass "B" 179	33·9 per cent. of Zn; 1·03 per cent. of Mn; 0·91 per cent. of Fe; 1·62 per cent. of Al; 1·75 per cent. of Sn; 0·78 per cent. of Pb; 1·01 per cent. of Ni
Bronze 183/1	5·17 per cent. of Zn; 5·01 per cent. of Sn; 3·51 per cent. of Pb; 0·51 per cent. of P; 0·51 per cent. of Ni; 0·24 per cent. of Sb; 0·14 per cent. of As
Bronze "C" 207	9·80 per cent. of Sn; 2·53 per cent. of Zn; 0·41 per cent. of Pb

CONCLUSIONS

Precipitation of cuprous thiocyanate from homogeneous solution by controlled reduction of cupric ions to cuprous with hydroxyammonium chloride provides a quick, simple, accurate and specific method for the determination of copper. The precipitates produced by this method have good filtration characteristics and in this respect are far superior to those produced by the classical heterogeneous precipitation procedures, notably in that they do not produce the fine suspension of cuprous thiocyanate that displays the phenomenon of creeping up the sides of apparatus in which it is handled.

Precipitation of copper in this way also provides a practicable alternative to electrolysis for removing copper from solutions before certain other metals are determined. It has been used for separating large amounts of copper from small amounts of iron, for the determination of traces of iron in analytical-reagent grade copper salts. It is hoped that this will form the basis of a further communication.

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The Determination of Magnesium in Calcium Salts

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A method is described for determining magnesium in the range 0 to 50 μg in calcium salts. After preliminary extractions of the sample solution at pH 11 (containing citrate and tartrate as masking agents) with 8-hydroxyquinoline and chloroform to remove interfering metals, the magnesium is extracted with 8-hydroxyquinoline in the presence of n-butylamine. The extracted magnesium is then determined spectrophotometrically at 380 mμ. The results show that there is no interference from calcium.

PUBLISHED specifications for analytical-reagent grade calcium salts commonly describe limit tests for magnesium based on its colour reaction with Titan Yellow (Clayton Yellow, Thiazole Yellow).[1,2,3,4] This reagent is not, however, particularly satisfactory for routine determinations of small amounts of magnesium in calcium salts. It forms a lake with magnesium, which will quickly precipitate unless stabilised with, for example, starch and glycerol. The colour reaction is performed in rather alkaline solution, so that the bulk of the calcium must be removed by a preliminary precipitation, for example, as calcium molybdate. The small amount of calcium remaining in the solution intensifies the colour of the magnesium - Titan Yellow lake, so that calcium should be added to each of the magnesium solutions used in the preparation of the calibration graph. Moreover, the quality of Titan Yellow varies from one supplier to another, and from batch to batch. It is a necessary precaution, therefore, to prepare a fresh calibration graph with every batch of Titan Yellow used.

Umland and Hoffmann[5] described the extraction of magnesium with 8-hydroxyquinoline (oxine) into chloroform in the presence of n-butylamine and tartrate. The magnesium in the organic extract was then determined directly by measuring its optical density at 380 mμ.

Calcium was not extracted by oxine and chloroform either in the absence or presence of butylamine. Umland and Hoffmann were thus able to determine the magnesium contents of calcium minerals in the range 0·28 to 18·2 per cent. of magnesium.

The magnesium was extracted as the ion-association system $[n\text{-}C_4H_9NH_3]^+$ $[Mg(ox)_3]^-$, where "*ox*" represents the anion of 8-hydroxyquinoline.

In the absence of butylamine, the normal magnesium oxinate, $Mg(ox)_2.2H_2O$, could not be extracted into chloroform. A preliminary extraction with oxine and chloroform in the absence of butylamine served to remove other metals, such as iron, that would interfere in the extraction of magnesium. (The organic phase from this preliminary extraction could be used to determine iron.)

Jankowski and Freiser[6] have used a similar procedure for determining microgram amounts of magnesium in the presence of milligram amounts of calcium, strontium and barium, with tartrate as a masking agent and tetra-alkyl ammonium salts to provide the cations of the extractable ion-pair.

We have adapted the method of Umland and Hoffmann to determine amounts of magnesium within the range 0 to 50 μg in the presence of up to 80 mg of calcium. The method could probably be applied to somewhat larger amounts of calcium.

EXPERIMENTAL

Umland and Hoffmann[5] extracted magnesium from a tartrate-containing solution having a pH between 10·5 and 11·5, which was obtained by adding ammonia solution. Tartrate was insufficiently effective to prevent precipitation of calcium with the amounts of calcium salts we wished to use. Precipitation was accompanied by low recoveries of added magnesium. Citrate proved to be an extremely effective masking agent, and in the presence of citrate it was found necessary to use sodium hydroxide to raise the pH of the solution to 11 or above. Tartrate was still added to the test solution, as we found that it prevented sudden large increases in pH, which occasionally caused localised precipitation in the vicinity of the added sodium hydroxide.

Preliminary experiments also showed that, under these conditions, two extractions with oxine and chloroform were necessary completely to remove more than about 10 μg of magnesium from the solution.

A 2 per cent. solution of calcium chloride was treated with citrate and tartrate, and its pH was adjusted to 11 with sodium hydroxide solution. It was then extracted several times with oxine and chloroform in the presence of n-butylamine to remove any magnesium present in the salt. It was then washed several times with chloroform to remove any remaining oxine.

Three portions of this solution, each containing 80 mg of calcium, were treated with 20, 30 or 40 μg of magnesium. The magnesium was then extracted by the procedure described below. The optical densities of the organic extracts were measured at 380 mμ. A parallel series of tests was carried out on solutions that contained no calcium. The optical densities obtained in the two series of tests are shown in Table I, and indicate that there is no interference caused by calcium.

TABLE I

COMPARISON OF OPTICAL DENSITIES OF MAGNESIUM COMPLEX EXTRACTED IN THE PRESENCE AND ABSENCE OF CALCIUM

Magnesium added, μg	Optical density of magnesium complex extracted from solutions containing—	
	no calcium	80 mg of calcium
20	0·380	0·380
30	0·565	0·575
40	0·765	0·760

METHOD

REAGENTS—

Analytical-reagent grade chemicals should be used when possible.

8-Hydroxyquinoline (oxine) solution—Dissolve 0·15 g of oxine in 100 ml of chloroform.

Butylamine solution—Dilute 20 ml of n-butylamine to 100 ml with water.

Potassium sodium tartrate solution, approximately M—Dissolve 28 g of potassium sodium tartrate in 100 ml of water.

Sodium citrate solution—Dissolve 20 g of trisodium citrate in 100 ml of water.

Sodium hydroxide solution, approximately M—Dissolve 4 g of sodium hydroxide pellets in 100 ml of water.

Chloroform.

PROCEDURE—

Dissolve a suitable weight of the calcium salt, containing not more than 50 μg of magnesium, in 10 ml of water and 6 ml of sodium citrate solution. Add 3 ml of potassium sodium tartrate solution, and adjust the pH of the solution to between 11 and 12 (measured with a pH meter) with sodium hydroxide solution. Transfer to a separating funnel, add 5 ml of oxine solution, shake vigorously for 5 minutes, allow to separate, and discard the organic layer. Extract again with 5 ml of chloroform, and again discard the organic layer. Add 1 ml of butylamine solution, and extract by shaking for 5 minutes with 5 ml of oxine solution. Run the organic layer into a dry vessel, add a further 1 ml of butylamine solution, and repeat the extraction with 5 ml of oxine solution. Combine the organic extracts, and filter the solution through a small funnel fitted with a glass-wool plug supporting a little anhydrous sodium sulphate into a 1-cm spectrophotometer cell.

Perform a blank determination on the reagents by the same procedure. Measure the optical density at 380 mμ of the test solution against the blank solution in the reference cell. Calculate the amount of magnesium in the sample from a previously prepared calibration graph.

NOTES—

1. Fifty milligrams of calcium sulphate, hydrated, dissolve easily in 6 ml of citrate solution.

2. Two hundred milligrams of calcium carbonate were dissolved in 5 ml of N hydrochloric acid and boiled. After 5 ml of water, 6 ml of citrate solution and 3 ml of tartrate solution had been added, about 2 ml of sodium hydroxide solution were required to raise the pH to between 11 and 12. When testing the other calcium salts, which did not require treatment with acid, only about 0·2 ml of sodium hydroxide solution was needed.

PREPARATION OF CALIBRATION GRAPH—

Dissolve 1·014 g of magnesium sulphate heptahydrate in 5 ml of 5 N sulphuric acid and sufficient water to produce 1 litre. Dilute this solution ten-fold immediately before use to give a solution containing 10 μg of magnesium per ml.

Measure from a burette 0-, 0·5-, 1·0-, 2·0-, 3·0-, 4·0- and 5·0-ml portions of the magnesium solution into small beakers. Dilute each solution to 10 ml with water, add 6 ml of sodium citrate solution, and continue as described under "Procedure." Plot a graph of optical density against micrograms of magnesium.

The graph is linear and passes through the origin. The molar extinction coefficient of the magnesium complex is about 5000.

RESULTS AND CONCLUSIONS

Known amounts of magnesium were added to solutions of calcium chloride (0·2 g of the hydrated salt) and calcium sulphate (50 mg of the hydrated salt). The magnesium was extracted and its optical density measured as described above, the original magnesium content of the salt being subtracted from the value obtained. The results of these experiments are shown in Table II.

TABLE II

RECOVERIES OF MAGNESIUM FROM SOLUTIONS OF CALCIUM SALTS

Sample	Magnesium added, μg	Magnesium found, μg
Calcium chloride (Batch "D") ..	5·0	5·0
	10·0	9·5
	20·0	19·2
	30·0	29·0
	40·0	38·0
Calcium sulphate (Batch "J") ..	20·0	20·0

Some typical results obtained by this method on AnalaR calcium salts are shown in Table III, together with the stated limit for magnesium.

TABLE III

TYPICAL MAGNESIUM CONTENTS OF ANALAR CALCIUM SALTS

Compound	Specified limit for magnesium, p.p.m.	Magnesium found, p.p.m.
Calcium carbonate	200	
Batch "A"		65·5
Batch "B"		45
Batch "C"		100
Calcium chloride, hydrated	200	
Batch "D"		15
Batch "E"		10
Batch "F"		12·5
Calcium chloride, dried	400	
Batch "G"		105
Batch "H"		75
Calcium sulphate, hydrated	1000	
Batch "J"		60

The procedure has been found suitable for determining small amounts of magnesium in calcium salts. It could easily be modified to deal with smaller or larger amounts of magnesium.

We thank the Directors of Hopkin & Williams Ltd. for permission to publish this paper.

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Application of the Uranyl Salt Method to the Determination of Arsenic by the Oxygen Flask Technique

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A modified oxygen flask technique is described for determining arsenic in organic compounds, the conventional platinum supports being replaced by aluminium spirals or steel gauzes, which resist attack by arsenic during the vigorous combustion.

A favourable gravimetric factor is ensured by the quantitative precipitation of the arsenic as ammonium uranyl arsenate and subsequent ignition, under controlled conditions, to the black triuranium octaoxide, U_3O_8. Phosphate and vanadate form similar insoluble ammonium uranyl salts, and the method is not directly applicable to organic compounds containing these elements without prior separation.

SEVERAL procedures are available for determining arsenic; titrimetric methods include the titration of tervalent arsenic by bromate[1] or iodate[2] in hydrochloric acid solution. A useful separation of arsenic from other elements can be achieved by reducing arsenic salts to elemental arsenic with consequent precipitation from solution. Hypophosphorous acid is the preferred reductant, and a titrimetric finish is generally employed, the precipitated arsenic being dissolved in standard iodine[3,4] or bromine.[5] The usual gravimetric method depends on the precipitation of quinquivalent arsenic as ammonium magnesium arsenate with subsequent ignition to the pyroarsenate.[6] According to Duval and Duval,[7] this compound is stable over the temperature range 415° to 885° C, but some arsenate may be reduced to the tervalent state and lost.[8]

Ammonium uranyl arsenate has an extremely low solubility product[9] ($1{\cdot}71 \times 10^{-24}$); this, and a favourable gravimetric factor, has made it the basis for the determination of arsenate arsenic.[10,11] Pullar[10] precipitated arsenate with uranyl ion and obtained a fine gelatinous precipitate soluble in mineral acids and insoluble in acetic acid, the determination being completed by careful ignition of the precipitate to uranyl pyroarsenate. Dupuis and Duval[12] do not consider this a suitable weighing form. Lewis and Davis[11] ignited the ammonium uranyl arsenate precipitate to triuranium octaoxide, an established weighing form, the arsenic being completely volatilised.

Tervalent arsenic compounds are volatile, and care has to be taken when destroying organic matter in organo-arsenical materials, this being usually accomplished by wet-oxidation methods.[11,13] The sealed nature of the oxygen flask technique makes it an obvious alternative, and it was first proposed for this purpose by Corner[14]; after combustion, most of the arsenic is in the tervalent state.[15] Belcher *et al.*[15] and Tuckerman *et al.*[16] do not consider the method adequate, as arsenic tends to alloy with the platinum supports and combustion is also unsatisfactory with silica spirals; they recommend wet combustion.

EXPERIMENTAL

PRECIPITATION OF AMMONIUM URANYL ARSENATE—

Lewis and Davis[11] precipitate ammonium uranyl arsenate at the boiling-point by adding uranyl acetate to a solution of arsenate in an acetic acid - ammonium acetate buffer, and then age the fine precipitate for some hours before collection by filtration.

In an attempt to produce a more compact and filterable precipitate we substituted ammonia derivatives for ammonia, adding them to boiling solutions of uranyl acetate in nitric - acetic acid. Precipitates were formed with diethylamine, ethylenediamine, guanidine carbonate and hydrazine hydrate, but these had no particular advantages over the ammonium derivative. However, slow precipitation with ammonia, generated by the hydrolysis of urea, under similar conditions yielded a compact coarsely crystalline and readily filterable precipitate, strikingly different from the much more intractable product of normal precipitation.

A suitable initial pH before the precipitation was found in the range 1·3 to 1·8; above pH 1·8 premature precipitation can occur, and below pH 1·3 the time of formation of the precipitate becomes too lengthy. In a study of the change in pH during the course of one precipitation, an initial drop in pH (from 1·7 to 0·9) was observed, the rate of decrease being most pronounced at the point when the bulk of the precipitate came down, after which the pH steadily increased as more ammonia was produced. This indicates that initially the homogeneously generated ammonia reacts to form ammonium uranyl arsenate and does not raise the pH as expected. Sodium and hydrogen ions were present, but the hydrogen and sodium uranyl arsenates are not preferentially formed, their solubility products being known to be $3{\cdot}17 \times 10^{-11}$ and $1{\cdot}35 \times 10^{-22}$, respectively,[9] *i.e.*, much greater than the value for the corresponding ammonium salt. Spectroscopic analyses of the ammonium derivative have confirmed that contamination by sodium ion is negligible.

In all the subsequent work, the precipitation was completed by continuing the hydrolysis until the pH was raised to 3·0 to 3·5 and then setting the solution aside for some hours before filtration at room temperature.

DRYING AND IGNITION OF THE PRECIPITATE—

Precipitates of $NH_4.UO_2.AsO_4.nH_2O$ prepared in the manner described, when collected on a porous porcelain crucible and dried in air, contained between 2·5 and 3·0 molecules of water. On drying these precipitates in an electric oven, most of this water was easily lost, the apparent degree of hydration depending on the temperature and time of drying. Gentle drying indicates the presence of a monohydrate, but this broke down and nearly anhydrous forms were always obtained. Thermogravimetric studies by Dupois and Duval[12] showed no stable plateau, and it is apparent that these hydrates are not good weighing forms. Their indefinite composition and hygroscopic nature make them of little analytical value, although they could be used for approximate work.

Further ignition of these precipitates, in electric ovens and furnaces, resulted in slight but continual losses in weight up to 400° C. At 500° C there was a sharp decrease in weight apparently corresponding to the formation of uranyl pyroarsenate, the weighing form used by Pullar, and this was accompanied by a change in colour of the precipitates from yellow to green. On raising the temperature to 800° C there was little, if any, change in weight, but the colour of the precipitates reverted to yellow. The weights of these ignited precipitates were always several per cent. below that calculated for $(UO_2)_2As_2O_7$, and this weighing form can be of no use for the exact determination of arsenic. It seems probable, from these observations, that evolution of ammonia reduces the precipitate; the colour changes indicate the presence of uranium^{IV}, and possibly arsenic is reduced to the tervalent state and so lost. Confirmation of this deduction was obtained by heating a sample of uranyl arsenate, previously dried at 220° C, in an ignition tube. Water and ammonia were expelled and a little white sublimate collected on the upper surface of the tube; at the same time the colour of the precipitate changed from yellow to green. The white sublimate was found to contain tervalent arsenic. The yellow uranyl pyroarsenate begins to decompose slowly when heated in an electric furnace at 850° to 900° C. This process is greatly accelerated by increasing the furnace temperature to 1200° C, but even at this temperature 16 hours' ignition is necessary to complete the decomposition to triuranium octaoxide. Petit and Kienberger[17] report prolonged ignition periods for the decomposition of uranium compounds to triuranium octaoxide, and, as this oxide loses a little oxygen at high temperatures (above 950° C), they recommend a final re-oxidation of the oxide by ignition at 850° C. We found it necessary to dissolve the ignited oxide in nitric acid, evaporate, and re-ignite at 850° C.

An entirely different pattern of behaviour was observed when the precipitate was collected on filter-paper and ignited over a gas burner. On gentle ignition over a low flame, just sufficient to burn off carbonised paper, a residue remained that was mainly black triuranium octaoxide contaminated by a little yellow uranyl pyroarsenate. (These observations are similar to those previously made by Lewis and Davis.[11]) It was found that the decomposition of the remaining amount of uranyl pyroarsenate was best effected by a further ignition in the reducing atmosphere of an enveloping gas flame from a large Amal burner. This was found to be a more satisfactory technique than the alternatives of adding powdered filter-paper or ammonium sulphate to the precipitate. As it is possible that this ignited oxide of uranium may contain some dioxide, complete oxidation to triuranium octaoxide was effected either by re-ignition in the oxidising conditions of an electric furnace (at 850° C) or by dissolving in nitric acid and re-igniting. (Care must be exercised when applying the latter technique, so that no loss occurs due to decrepitation.) It was also found possible to carry out a gas-ignition procedure after collecting the precipitate in a porous porcelain crucible, the reducing gases from the burner permeating the porous base of the crucible and effecting reduction.

It is clear from these observations that reducing conditions greatly facilitate the ignition of the precipitate to triuranium octaoxide, and presumably reduction of arsenate to arsenious oxide or arsine occurs, with the consequent loss of arsenic. The sensitivity of uranyl pyroarsenate to a reducing atmosphere makes it an unreliable weighing form.

Results of these tests of reliability and accuracy on the gravimetric procedures are tabulated in Tables IA and IB, and show that both uranyl pyroarsenate and ammonium uranyl arsenate as weighing forms have little analytical value, although the latter could be used for approximate work. Weighing the precipitate as triuranium octaoxide, after ignition by either of the described procedures, yielded good results. For this procedure it was found preferable to collect the precipitate on a No. 44 filter-paper and ignite in a small silica or recrystallised alumina crucible (10 ml). The occasional low results when a porous porcelain crucible was used, were probably due to mechanical loss, the fine oxide being carried away by the gas passing through the porous base.

TABLE IA

APPARENT RECOVERY OF ARSENIC FROM DIFFERENT WEIGHING FORMS

Arsenious oxide was used as standard and the amount of arsenic present in each test was 25·00 mg

Arsenic, weighed as $NH_4.UO_2.AsO_4$, mg	Drying temperature, °C	Drying time, hours	Arsenic, weighed as $(UO_2)_2.As_2O_7$, mg	Arsenic, weighed as U_3O_8, mg
25·3	110	2	24·1	24·99
25·4	110	16	24·1	-
25·2	110	16	·	24·79
25·5	110	16	—	24·99
26·0	110	2	—	—
25·3	110	16	·	24·97
25·2	125	16	24·7	24·81
24·8	125	16	24·2	24·88
25·8	125	2	24·6	-
25·1	160	2	24·3	—
25·3	160	2	24·2	--

TABLE IB

FURTHER RESULTS

In a series of further experiments with 25·00 mg of arsenic (again as arsenious oxide) the results obtained by the uranyl acetate precipitation method, with subsequent direct ignition to U_3O_8, were—

Arsenic found, mg	24·88, 24·95, 25·01, 25·00, 25·01, 25·08, 24·98
Mean, mg	24·987
Standard deviation, mg	0·057
Coefficient of variation, % ..	0·022

FACTOR FOR THE CONVERSION OF TRIURANIUM OCTAOXIDE TO ARSENIC—

The sample of uranyl acetate used in this work was not of natural isotopic composition. The atomic weight of uranium of natural isotopic composition is 238·07 and consists of a mixture of three isotopes, ^{238}U, ^{235}U and ^{234}U. The physical atomic weight of pure isotope, ^{238}U, is 238·12493, and uranyl compounds supplied by the manufacturers will be enriched in this isotope. In calculating a factor we took the atomic weight of uranium as 238·10, as the results below show that the effect on the factor is slight—

Atomic weight of uranium	238·07	238·10	238·12
Conversion factor of U_3O_8 to arsenic	0·26683	0·26680	0·26677

The stoicheiometric formulation for the corresponding uranyl phosphate (prepared by the direct ignition of ammonium uranyl phosphate) as $(UO_2)_2.P_2O_7$ has recently been criticised by Wright, Hayes and Ryan.[18] As a result of a detailed investigation they suggest that the more correct formula is $(UO)(UO_2).P_2O_7$, which contains both quinquivalent and sexavalent uranium. This criticism obviously does not affect the work described here, as we use the oxide as the final weighing form.

OXYGEN FLASK COMBUSTION—

Previous workers using platinum or silica supports for the sample material have found the oxygen flask technique unsatisfactory for determining arsenic in organic compounds. Our preliminary observations confirmed this; with a sample of *o*-arsanilic acid, we found unmistakable signs of attack on platinum gauzes, which were rendered brittle. Substitution of glass spirals for the platinum gauze did not solve the problem, as combustions were smoky and carbon was deposited on both the spiral and the flask, and results were low.

It was decided to try other metallic supports in the hope that they might prove more satisfactory than platinum. For this purpose the stopper of the oxygen flask was fitted with a glass hook, so that spirals and gauzes of various materials could be hung from it. Oxidised copper spirals were first used in the hope that the oxide film would assist combustion and prevent reduction to arsenic, but, despite excellent combustions, poor results were obtained, again, presumably, because of alloy formation (there were some signs of attack on the copper).

The use of steel gauzes and aluminium spirals gave much improved results, there were no signs of attack on these materials (although the aluminium tended to melt) and combustion was adequate. Steel spirals gave good combustions and aluminium less so; with aluminium it was necessary to use tissue rather than filter-paper and get the combustion going well initially. After dissolution of the white sublimate of arsenic oxides, which were formed on the sides of the flask, and subsequent acidification and oxidation to arsenate with bromine water, the arsenic was determined as previously described. The results of these preliminary investigations are tabulated in Tables II and III. Further results obtained with steel and aluminium supports gave satisfactory recoveries of arsenic from *o*-arsanilic acid, arsenious oxide and acetarsol.

METHOD

APPARATUS—

Oxygen combustion flask, 1 *litre, flat-bottomed.*

Sample supports—(*a*) A 6-cm × 2-cm rectangle of mild-steel gauze (30 meshes to the lineal inch), folded across its length 2 cm from one end; (*b*) 1-mm diameter aluminium wire.

Sample containers—A folded No. 44 filter-paper or tissue paper, 2 cm square, with a tail to act as a touch paper.

REAGENTS—

Precipitating solution—A solution containing 2 per cent. w/v of uranyl acetate dihydrate, 12·5 per cent. w/v of urea, 20 per cent. v/v of glacial acetic acid and 0·5 per cent. v/v of concentrated nitric acid.

PROCEDURE—

Weigh out about 40 mg of the organic arsenical sample. Either wrap it in a tissue paper and bind with aluminium wire or place in a shaped No. 44 filter-paper and secure in a mild-steel gauze. Attach to the hook fused on to the glass stopper of the oxygen flask. Fill the oxygen flask with oxygen (this can be ensured by displacement of water), and add 40 ml of 1·25 per cent. w/v sodium hydroxide solution. Ignite the touch paper, and quickly insert the stopper into the flask, carefully tilting the flask so that the arsenical sublimate condenses on the side of the flask rather than on the neck and stopper. Set the flask aside for 2 to 3 hours so that the mist formed during combustion is cleared. Wash down the spiral or gauze with water, and remove from the hooked stopper. Replace the stopper; and shake the flask for 5 minutes. Filter the contents into a 150-ml beaker, acidify with 4 N nitric acid to the red colour of thymol blue indicator, and oxidise arsenite to arsenate with bromine water, adding a 5-ml excess. Add 20 ml of precipitating solution, and raise the temperature of the solution to between 80° and 90° C. A precipitate should form within a few minutes.

Maintain the temperature until the pH of the solution reaches 3·0 to 3·5, as indicated by the green colour of the bromophenol blue indicator. Set aside for some hours at room temperature.

Filter through a No. 44 filter-paper, and wash the precipitate with 0·5 per cent. v/v acetic acid. Transfer the filter-paper to a 10-ml fused alumina crucible, carefully burn off the filter-paper over a bunsen burner, and then ignite in the enveloping flame of an Amal burner for 1 to 2 hours. Alternatively, place the crucible in a cold electric furnace, burn off the filter-paper at 600° C, and increase the temperature to 1200° C; maintain this temperature overnight. Dissolve the ignited oxides in a few drops of concentrated nitric acid, carefully evaporate to dryness, and re-ignite by raising the temperature to 850° C in an electric furnace. Weigh as U_3O_8.

TABLE II

RECOVERY OF ARSENIC AFTER OXYGEN FLASK COMBUSTION WITH VARIOUS SUPPORTING MATERIALS

o-Arsanilic acid was the organic compound used in these recovery experiments

Support material	Arsenic added, mg	Arsenic recovered, mg	Observations
Platinum gauze	11·16	7·52	Platinum pitted and swollen. Good combustions
Glass spiral	11·02	9·98	Poor combustion, smoke and carbon deposits
	14·65	13·26	
Copper spiral	17·32	13·75	Good combustions. Copper melts
Copper gauze	14·03	7·12	
	14·01	6·92	
Aluminium spiral	14·29	14·28	Moderate combustions. Aluminium melts
	14·11	13·77	
Stainless-steel gauze	16·02	15·90	Good combustions
	13·92	13·53	
	14·04	13·74	

TABLE III

RECOVERY OF ARSENIC FROM VARIOUS COMPOUNDS WHEN STEEL OR ALUMINIUM SUPPORTS WERE USED

Support material	Arsenical compound	Arsenic (calculated), mg	Arsenic found, mg
Aluminium spiral	Arsenious oxide	16·04	16·08
		15·50	15·60
Stainless-steel gauze	Arsenious oxide	15·76	15·88
Mild-steel gauze	Arsenious oxide	15·71	15·74
Aluminium spiral	*o*-Arsanilic acid	14·29	14·28
		14·11	13·77
		14·08	14·10
		13·87	13·70
Stainless-steel gauze	*o*-Arsanilic acid	16·02	15·90
		13·92	13·53
		14·04	13·74
Mild-steel gauze	*o*-Arsanilic acid	14·49	14·49
		14·13	13·85
		15·63	15·23
Aluminium spiral	Acetarsol	11·29	11·28
		12·06	12·04
		11·48	11·04
Mild-steel gauze	Acetarsol	11·20	11·18
		11·18	10·81

CONCLUSION

The work of Lewis and Davies[11] is confirmed and extended. Arsenic can be accurately determined by controlled precipitation as ammonium uranyl arsenate and subsequent ignition to U_3O_8 under the conditions described in this paper. Metals forming insoluble arsenates, hydroxides or acetates in the stipulated pH range will obviously interfere and must be removed by conventional methods. Phosphates and vanadates form similar ammonium uranyl salts, but on ignition give the corresponding pyrophosphate and pyrovanadate compounds, respectively.

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Determination of Low Concentrations of Sodium Dodecylbenzenesulphonate

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A brief review is given of methods of analysis in which a complex formed between a dyestuff and a surface-active agent of opposite polarity is extracted into an organic liquid in which the dyestuff itself is insoluble.

Two new methods are described in which this technique is used; they are suitable for the determination of anionic surface-active agents at low concentration. The first, in which an excess of magenta is added and the optical density of the organic layer is measured, can be used at concentrations down to 10^{-7} M. The second, in which magenta is titrated directly against surface-active agent, and either the visually assessed complete transfer of colour to the organic phase or the transfer to equal colour intensity in both phases is used as end-point, is satisfactory down to a concentration of 5×10^{-7} M. The influence of salting-out effects is discussed.

DURING a recent investigation into the adsorption of surface-active agents on mineral powders it became necessary to develop a rapid and simple method for determining sodium dodecylbenzenesulphonate in the concentration range 10^{-5} to 10^{-7} M in solutions whose total ionic concentrations varied from 10^{-7} to 10^{-1} M. A search of the literature revealed several possible methods, and they were examined to see if they were suitable or could by modification be made suitable for the purpose. Methods have been described[1,2] in which a complex formed between a dyestuff and a surface-active agent of opposite polarity is extracted into an organic liquid in which the dyestuff itself is insoluble; the extract is then examined spectroscopically.[3] A quicker and more convenient method is the direct titration of anionic - cationic surface-active agents proposed by Hartley and Runnicles.[4] Their method made use of the colour change of bromophenol blue to determine the end-point.

Epton[5] and Barr, Oliver and Stubbings[6] introduced the idea of two-phase titration, which combined anionic - cationic titration with extraction of the coloured complex. Epton's method consists in shaking the unknown anionic surface-active agent with methylene blue and chloroform. The cationic surface-active agent used as titrant reacts preferentially with the unknown anionic surface-active agent until the reaction is complete, after which further addition of titrant reforms the water-soluble salt of the dye. With this system three end-points are possible, *viz.*—

(*a*) the first appearance of dye in the aqueous phase;

(*b*) the complete transfer of dye to the aqueous phase;

(*c*) the partial transfer of dye to give equal coloration in both layers.

If the titration is performed in the reverse manner, that is, with the anionic surface-active agent as titrant, two more end-points are possible, *viz.*—

(*d*) the first appearance of colour in the chloroform;

(*e*) the complete transfer of dye to the chloroform.

Barr, Oliver and Stubbings found that bromophenol blue was a more suitable indicator for end-point (*d*).

Edge,[7] who used bromophenol blue, found that sodium dodecyl sulphate could be titrated successfully to end-point (*b*) with cetyltrimethylammonium bromide at concentrations down to 1×10^{-5} M. The presence of electrolytes, however, caused a stable emulsion to be formed at the end-point. A disadvantage of end-points (*b*), (*c*) and (*e*) is that the coloured complex transferred to the non-aqueous layer is formed from the surface-active agent and the dyestuff. Therefore, particularly at low concentrations, it is essential that the concentration of dyestuff and the amount of it added in each titration should be exactly the same as those used in constructing the calibration curve.

Edge,[7] and later Kemp and Nutt (personal communication), used Barr, Oliver and Stubbing's original system and end-point by titrating cetyltrimethylammonium bromide with sodium dodecyl sulphate and bromophenol blue as indicator. It was found that, after the solution had been shaken, the first colour to cross the interface was in the form of concentrated blue wisps that were easily detectable. By taking the appearance of these wisps as the end-point, it was found possible to determine concentrations as low as 1×10^{-5} M. Further investigation of this system for application in the concentration range in which we were interested showed it to be unsatisfactory, since the wisps of colour were barely visible.

Other investigators have used rosaniline and pararosaniline for the colorimetric determination of long-chain alkyl sulphates. Karush and Sonenberg[8] used these dyes in 0·025 M phosphate buffer and extracted the dye - surface-active agent complex with a mixed 50 per cent. chloroform - 50 per cent. ethyl acetate solvent. Some difficulty was experienced, since transfer of dye to the organic phase occurred before the surface-active agent was added. The method was found to be sufficiently sensitive for determining concentrations of 5×10^{-6} M with an error of about 2 per cent.

Wallin[9] used basic fuchsin as the dye for the colorimetric determination of sodium dodecylbenzene sulphate. Basic fuchsin consists of rosaniline and pararosaniline, but Wallin states that it is not believed that both components take part in the reaction. The dye is insoluble in chloroform, so that chloroform can be used as a blank. The fuchsin does, however, react to produce a chloroform-soluble complex with several salts, namely nitrates of alkali metals on the acid side, potassium iodide and bromide, and calcium and sodium chlorides. It appears that the transfer of dye noted by Karush and Sonenberg may have been due to their use of the phosphate buffer. Wallin was able to detect 0·5 mg of surface-active agent in 20 ml of extractant.

Preliminary experiments showed that the detection of the coloured wisps could be improved by selecting a dyestuff having an intense colour and a high interfacial activity. Dyestuffs that transferred from one phase to another during the shaking (emulsification) procedure were unsuitable for use in Barr, Oliver and Stubbings's method. Several dyestuffs were tried, and it was finally decided to use magenta (rosaniline) for the determination of sodium dodecylbenzenesulphonate.

EXPERIMENTAL

REAGENTS—

The aqueous solution was prepared by triturating a portion of magenta powder with a little water in an agate mortar, and then washing it into a beaker and adjusting the volume to 200 ml. An equal volume of hot water was added, the solution was stirred and then set aside for 3 days. The supernatant liquor was decanted and again diluted with an equal volume of hot water. This solution was then stored as a stock solution. A working solution was prepared by accurately diluting a portion of the stock solution to one fifth of its concentration.

During the period of the investigation, it was found that the stock solution had deposited some material and formed a stable froth after it had been shaken. The deposited material was removed by siphoning off the liquid from below the surface. A further working solution was made up and was found to have the same concentration as the original working solution.

Standard 10^{-3} M solutions of sodium dodecylbenzenesulphonate were normally made up weekly; weaker solutions were prepared daily by dilution of the 10^{-3} M solution, an Agla micrometer-syringe pipette being used when 0·5 ml or less was required. A 10^{-3} M solution of sodium dodecylbenzenesulphonate is stable for a fortnight, loss by decomposition and slow adsorption being negligible at this concentration, whereas a 10^{-6} M solution decomposes in a few days and a 10^{-7} M solution is apparently completely decomposed overnight.

CALIBRATION OF SPECTROPHOTOMETER READINGS—

Initially, the spectrophotometric technique was explored, the method used being similar to that described by Few and Ottewill.[3]

A calibration curve was obtained for the spectrophotometer (Unicam SP600) by the procedure described below. Analytical-reagent grade chloroform (5 ml) and 5 ml of standard surface-active agent solution were put by pipette into a glass-stoppered test-tube with an excess of saturated magenta solution; one drop from a dropper was usually sufficient. The tube was shaken 50 times and then set aside for about 5 minutes to allow the phases to separate. The chloroform was then carefully transferred by pipette to a 1-cm cell, and the cell was placed in the spectrophotometer. A further 5 minutes were allowed for the water droplets produced during the transfer of the chloroform to disperse, and then the optical density was measured at 550 mμ against chloroform. This procedure was repeated at different concentrations of surface-active agent, and the curve shown in Fig. 1 was prepared.

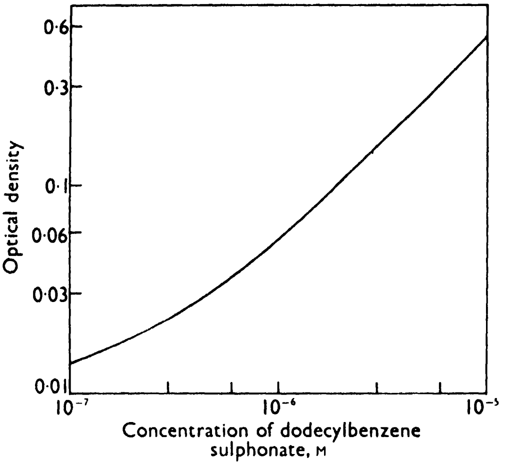

Fig. 1. Graph showing relationship between optical density and concentration of dodecylbenzenesulphonate

The method was compared with that of Longwell and Maniece[2] at the Water Pollution Research Laboratory, and the results (personal communication from E. G. Eden) showed that in speed and simplicity it had advantages over Longwell and Maniece's procedure, but it gave slightly higher values and was insensitive at concentrations of surface-active agent above 5 p.p.m. No examination was made of the effect of impurities on the results by the method. Abbott[10] has modified Longwell and Maniece's method and has made a careful study of the effects of impurities at a constant ionic concentration of about 0·01 M. As shown later, the amount transferred between phases depends on ionic concentration, but the difference in emphasis of the analytical errors is entirely due to the different objects of the investigations.

TWO-PHASE TITRATION—

Further investigation into the possibilities of the two-phase anionic - cationic titration was carried out. The first appearance of wisps of colour in the chloroform was found to be unsuitable as an end-point. As the magenta itself reacts with the anionic surface-active agent to form the chloroform-soluble complex, it was decided to titrate solutions containing known volumes of the working magenta solution with standard surface-active agent. Titrations were carried out in 60-ml glass vials with polythene stoppers. Adsorption of the dye on the surface of the glass was reduced to a minimum by treatment with Silicone Fluid MS1107. Equal volumes (25 ml) of chloroform and water were used for the titrations, so that end-points (*c*) and (*e*) could be studied. Small portions of the working magenta solution were added from an Agla micrometer-syringe pipette, and the sodium dodecylbenzenesulphonate solution was added from a 5-ml burette. Normally, three titrations were carried out for each different concentration of magenta, and, after each titration, the optical densities of the chloroform phase and aqueous phase were measured. These results are shown in Table I as average values for each series of three determinations.

It was found that the end-point showing equal colour in both phases was easily detected in daylight and in yellow-green artificial light from a microscope lamp, provided that more than 0·05 ml of magenta solution was present. Titrations at this low concentration were successfully carried out in bright daylight only; even then the results showed more

TABLE I

TITRATION OF MAGENTA SOLUTION WITH SODIUM DODECYLBENZENESULPHONATE

Volume of magenta solution, ml	Volume of 10^{-4} M surface-active agent, ml	Volume of surface-active agent per 0·1 ml of magenta solution, ml	Optical densities		End-point
			Chloroform phase	Aqueous phase	
0·25	0·79	0·316	—	—	Partial transfer of dye
0·25	0·86	0·344	0·199	0·174	
0·15	0·52	0·347	0·130	0·108	
0·15	0·51	0·340	0·117	0·104	
0·05	0·128	0·256	0·029	0·036	
0·05	0·155	0·310	0·036	0·032	
0·25*	0·79	0·316	—	—	
0·15*	0·50	0·333	—	—	
0·25	1·89	0·756	0·387	0·013	Complete transfer of dye
0·15	1·14	0·760	0·231	0·009	
0·15	1·07	0·713	0·234	0·012	
0·05	0·293	0·586	0·065	0·008	

* Results obtained with freshly diluted magenta solution.

scatter than those obtained at higher concentrations. Complete transfer of the dye was a little more difficult, since the aqueous phase always appeared faintly pink, probably from scattered light from the underlying coloured chloroform phase. In this titration artificial light was found to be of no use.

It can be seen from column 3 of Table I that the volume of surface-active agent solution required to the half-way end-point was about 0·3 ml per 0·1 ml of magenta solution. The concentration of the surface-active agent solution was 10^{-4} M, and this was diluted to 25 ml for the titration. Thus surface-active agent concentrations of about 10^{-6} M were in fact successfully titrated.

SPECTROPHOTOMETRIC INVESTIGATION OF THE TWO-PHASE METHOD—

To check on the quantitative transfer of the dye complex into the chloroform phase, equal amounts of chloroform and water (25 ml) were shaken with 0·25 ml of magenta solution and measured amounts of 10^{-4} M sodium dodecylbenzenesulphonate. After the extraction, the optical densities of the chloroform phase and of the aqueous phase were measured spectrophotometrically; the results are shown graphically in Fig. 2. No allowance has been made for the increase in volume of the aqueous phase. It can be seen that a linear relationship

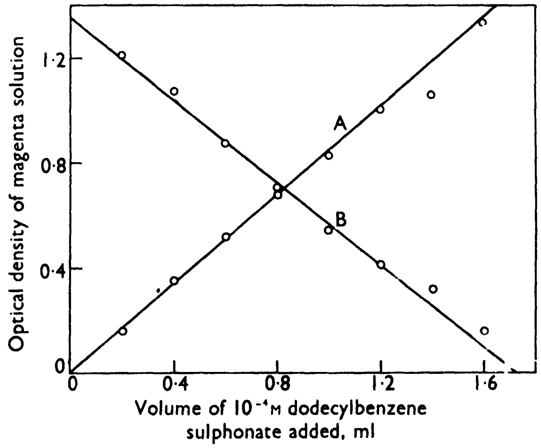

Fig. 2. Graphs of titration of magenta solution with 10^{-4} M dodecylbenzenesulphonate: curve A, chloroform solution; curve B, aqueous solution

exists between the concentration of surface-active agent and the amount of dye transferred to the chloroform. The point of intersection of the lines, corresponding to 0·83 ml of 10^{-4} M surface-active agent, agrees reasonably well with the titration figures obtained previously for the half-way end-point. Spectrophotometric determination of the end-point indicated by complete transfer of dye was always found to be slightly more than double the concentration at which optical densities of the phases were equal, and this was also usually so when the end-points were determined visually.

EFFECT OF NON-SURFACE-ACTIVE AGENTS—

The effect of ionic concentration on the transfer of magenta from the aqueous to the organic phase was studied with potassium chloride. Equal volumes (25 ml) of chloroform and potassium chloride solution of known concentration were shaken with 0·25 ml of magenta solution. Considerable transfer of magenta into the chloroform was observed. In two tests, 2×10^{-8} and 4×10^{-8} moles of sodium dodecylbenzenesulphonate were added; the optical densities are shown in Table II. It appears that, for a constant ionic concentration, there would be a constant displacement of the optical density.

TABLE II

SALTING-OUT OF MAGENTA WITH POTASSIUM CHLORIDE

Concentration of potassium chloride, M	Sodium dodecylbenzene-sulphonate present, moles $\times 10^8$	Optical density of chloroform phase
1·0	0	0·900
0·2	0	0·386
0·1	0	0·252
0·1	2	0·376
0·1	4	0·544

Since Wallin had noted complex formation between basic fuchsin and several salts, it was considered necessary to investigate more fully the transfer of magenta in the presence of potassium chloride. Twenty-five millilitres of 0·1 M potassium chloride and 1 ml of 0·003 M magenta were repeatedly extracted with portions of chloroform until no further transfer of dye could be detected; the aqueous layer was faintly coloured with magenta. The concentration of dye was then made up to approximately its original value, and a further extraction with chloroform was carried out. The amounts of magenta transferred during this extraction and the first extraction were found to be the same. This shows that the transfer is not due to impurities in the magenta nor to small amounts of surface-active agent in the potassium chloride.

A direct test was then made to distinguish between salting-out effect and complex formation by measuring the conductivities of dilute solutions of potassium chloride and magenta. The results were—

(*a*) specific conductance of 10^{-3} M magenta solution was $2·46 \times 10^{-4}$ mho per cm;

(*b*) specific conductance of $3·84 \times 10^{-3}$ M potassium chloride was $5·51 \times 10^{-4}$ mho per cm;

(*c*) specific conductance of saturated magenta solution in $3·84 \times 10^{-3}$ M potassium chloride was $7·87 \times 10^{-4}$ mho per cm.

Adding (*a*) and (*b*) gives $7·97 \times 10^{-4}$ mho per cm, which is about the expected value of (*c*) if there were no loss of ions due to complex formation. It appears, therefore, that the transfer of magenta in the presence of potassium chloride is a salting-out effect and is not due to formation of a chloroform-soluble complex.

The salting-out of some other dyes was checked by extracting the dye with chloroform in the presence of 0·1 M potassium chloride. Large amounts of malachite green were found to be transferred in the presence of the salt. Methylene blue showed some transfer, but with bromophenol blue the transfer was negligible. With rhodamine B the colour disappeared from both the aqueous and chloroform phases after shaking.

CONCLUSIONS

The appearance of the water - organic liquid interface has proved critical when assessing end-points in the determination of surface-active agents by the two-phase titration of anionic against cationic reagent with a dye as indicator that forms a complex with one reactant and can transfer from one phase to the other. Visual detection of these effects, however, becomes difficult at concentrations of surface-active agent of less than 10^{-5} M.

In the two methods of analysis studied here the dye has been used as titrant rather than as an indicator. That is, the positively charged dye magenta has been used for determining an anionic surface-active agent, but still by the two-phase technique. These methods should be applicable to other anionic surface-active agents.

The spectrophotometric technique, in which an excess of magenta is added and then the optical density of the organic phase is measured, can be used down to a concentration of 10^{-7} M. The direct titration of magenta against surface-active agent, in which either the visually assessed complete transfer of colour to the chloroform or transfer to equal colour intensity in both phases can be used as end-point, is satisfactory down to 5×10^{-7} M. In this method the "half-way" end-point is the best.

Magenta has a disadvantage in that its transfer is dependent on ionic concentration. In solutions of 0·01 M sodium fluoride the amount of magenta extracted into the chloroform phase in the absence of surface-active agent was just measurable spectrophotometrically. In the presence of higher concentrations of potassium chloride, 0·1 to 1·0 M, considerable amounts of dye were transferred to the chloroform before the addition of the surface-active agent. Although this point was not investigated, it would appear that, for solutions of high ionic concentration, the spectrophotometric method could still be used provided that measurement was made against a cell containing a blank solution prepared by shaking chloroform with the ionic solution containing magenta but no reagent.

The transfer of some dyes, in the presence of different electrolytes, has been found to be due to the formation of chloroform-soluble complexes. It has been shown in this investigation that the effect of potassium chloride on a magenta solution is a salting-out of the dye. It is noted from the literature, however, that magenta does form a complex with silver ions. This effect was noticed when attempts were made to determine concentrations of anionic surface-active agents in solutions containing approximately 10^{-5} M silver fluoride.

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The Determination of Silicon, Zinc and Magnesium in Gallium Arsenide by Neutron-activation Analysis

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The use of short-lived isotopes in the determination of trace amounts of impurities in gallium arsenide is described. Methods are presented for determining silicon, zinc and magnesium. The effect of thermal neutron self-shielding in the determination of silicon and magnesium has been investigated and shown to be insignificant.

NEUTRON-activation analysis is a useful technique for the study and quantitative measurement of trace amounts of impurities in highly purified materials, such as those used in the semiconductor field.[1] When the host material consists of elements of high thermal neutron cross-section, a considerable amount of undesired activity is induced on irradiation. If the activities induced in the host material are short-lived compared with those induced in trace constituents, samples may be allowed to cool before the determinations of trace amounts of impurities are carried out, *e.g.*, the determination of arsenic (^{76}As, $t_{\frac{1}{2}} = 26{\cdot}7$ hours) in silicon (^{31}Si, $t_{\frac{1}{2}} = 2{\cdot}62$ hours).[2] When the activities in the host material are longer lived than those produced in the trace constituents, considerable radiochemical purification (with high decontamination factor) may be required, and the early stages of such procedures may have to be performed in shielded facilities, with associated difficulties. When it is possible to measure the trace amounts of impurities by activation to short-lived species, the short irradiation times required bring a reduction in the activity induced in the host material, and with it a reduction in the radiation associated with handling the material.

For the determination by neutron activation of trace amounts of impurities in gallium arsenide, the fast-transfer facility of the Merlin reactor[3] has been used. The fast-transfer facility, a pneumatic tube for the transfer of samples from the laboratory to the reactor core, is a feature frequently incorporated in the design of research reactors. At a reactor power of 5 MW the thermal neutron flux at the core terminus of the fast-transfer facility was 4×10^{13} neutrons per sq. cm per second.

Methods have been developed for determining silicon, zinc and magnesium in gallium arsenide. The half-lives of the active species measured were 2·62 hours, 52 minutes and 9·5 minutes, respectively. The separation of these species requires radiochemical purification procedures that must be completed in a time similar to the half-life of the species separated. The development of these procedures is described below.

All chemical operations were performed in a fume cupboard with a shielding of 2 inches of lead. An upper limit of 100 millicuries of activity to be handled was set, for reasons of radiological protection. For the determination of silicon by irradiation, this limited the size of gallium arsenide samples to approximately 100 mg, which was the size usually taken for the determinations of zinc and magnesium.

The final precipitates obtained in the chemical procedures were mounted on standard aluminium planchettes, and the decay of the radioactive species was followed with a 1-inch diameter Geiger - Müller counter. A scintillation counter with a hundred-channel pulse-height analyser was used for the rapid identification of contaminating activities when the procedures were being developed.

When the host material and trace impurity differ by only 1 or 2 units of *Z*, the effect of fast neutrons on the host material may lead to the emission of charged particles, with the production of other active species of the trace constituent. This has been found to occur in the determination of zinc in gallium arsenide, and a measurement of the magnitude of this interference is given under "Determination of Zinc," p. 524.

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When the neutron cross-sections of elements in the host material are large, the neutron flux through a specimen may be considerably attenuated giving a reduced activation of impurities inside the sample, and hence an erroneously low content of impurity. The effect of such neutron self-shielding for silicon and magnesium in gallium arsenide has been investigated.

EXPERIMENTAL

DETAILS OF IRRADIATIONS—

Samples of gallium arsenide were contained in polythene ampoules, which were placed in an epoxy-resin carrier and transferred to the core of the reactor by the fast-transfer facility. The build-up of long-lived activities in the matrix material was kept to a minimum by limiting the length of irradiation to a time equal to the half-life of the species to be separated. After irradiation, the sample was returned to the laboratory by the fast-transfer facility (transit time 8 seconds) and transferred to a shielded cell for processing.

DISSOLUTION OF SAMPLES—

Dissolution of gallium arsenide in aqua regia has been found to take 10 to 15 minutes for a 100 mg specimen. When short-lived species are being determined, this time would result in a decrease in the sensitivity of the method. The use of electrolytic dissolution has been found to aid the solution of pure gallium in nitric acid.[4] This method has been found applicable to gallium arsenide, and it was possible to dissolve 100-mg samples of gallium arsenide in nitric acid on passing a current of 1 to 2 amps at 15 volts for 3 to 4 minutes.

DETERMINATION OF SILICON

It has been found possible completely to remove arsenic activity from silicon by precipitation of silica with ammonium carbonate and thorough washing of the precipitate. Gallium hydroxide is also precipitated by this procedure, and complete removal of gallium activities from silicon has been found difficult to achieve. With 14·1-hour gallium-72 as tracer, the effectiveness of different procedures in removing gallium from silicon was investigated. Although most of these methods led to separation factors of 10^4 or better, when they were tried sequentially little increase in separation factor was obtained. This may be attributed to the association of gallium and silica as a radio-colloid that was not dissolved by the hydrofluoric acid used in the intermediate treatment. Use of sodium hydroxide solution as the dissolving medium in the intermediate treatment has overcome this difficulty.

Two gravimetric compounds for the determination of chemical yield have been investigated; silica and quinoline molybdosilicate.[5] The latter is the easiest to form in stoicheiometric proportions, but suffers from the disadvantage of having an extremely low silicon content. Investigations suggest that this leads to approximately 50 per cent. absorption of silicon-31 β^- particles in the precipitate, which is not desirable. For these reasons, silica has been used as the final gravimetric compound for the determination of chemical yield and for counting.

Sources of silica prepared from irradiated gallium arsenide were found to contain a contaminating activity with a half-life of 82 minutes. This was identified as germanium-75, produced by the fast-neutron reaction ^{75}As (n,p) ^{75}Ge, and this impurity was eliminated by adding germanium carrier at the dissolution stage and the removal of germanium with gallium in the subsequent procedure.

The complete radiochemical procedure for separating and purifying silicon from gallium arsenide is described below. After dissolution of gallium arsenide in a solution of silicon and germanium carriers in hydrofluoric - nitric acid, silica, germanium hydroxide and gallium hydroxide were precipitated with ammonium carbonate. The precipitate was washed, and dissolved in sodium hydroxide solution. Silica was precipitated by heating to fumes with perchloric acid. Dissolution and precipitation of the silica was repeated, with the addition of holdback carriers for gallium and germanium. The silica was redissolved and converted to the yellow molybdosilicate complex, which was extracted into pentanol and back-extracted into sodium acetate solution.[6] The complex was destroyed by heating with perchloric acid, and silica was precipitated. Molybdenum trioxide, which was also precipitated at this stage, was leached from the precipitate with ammonia solution. The precipitate was then washed with ammonia solution, water and alcohol, and mounted on an aluminium planchette for counting and for determining the chemical yield.

The processing of a single sample was found to take approximately 2 hours. The performance of replicate determinations side by side was considered inadvisable in view of the high activity levels associated with irradiated gallium arsenide.

PROCEDURE—

Transfer the irradiated gallium arsenide (up to 100 mg) to a dissolution cell containing 1 ml of concentrated nitric acid, 5 mg of silicon in hydrofluoric - nitric acid mixture and a few milligrams of germanium carrier. Dissolve the gallium arsenide by passing a current of 1 to 2 amps at 15 volts for 3 to 4 minutes.

Transfer the solution to a 50-ml beaker containing 5 g of ammonium carbonate, 2 ml of ammonia solution and 5 ml of water. Rinse the cell with 2 ml of water, and add the rinsings to the contents of the beaker. Boil for 10 minutes to complete the precipitation of silica, gallium hydroxide and germanium hydroxide. Spin in a centrifuge, and discard the supernatant liquid. Wash the precipitate with a 10 per cent. w/v solution of ammonium carbonate, and discard the washings.

Dissolve the precipitate by warming with 6 to 8 drops of 6 N sodium hydroxide, and transfer to a 10-ml beaker with the aid of 1 ml of water. Add 0·5 ml of perchloric acid, and heat until fumes are evolved to precipitate silica. Digest the precipitate with 5 ml of 6 N hydrochloric acid, spin in a centrifuge, and discard the supernatant liquid. Wash the precipitate with a further 5 ml of 6 N hydrochloric acid, and discard the washings.

Dissolve the precipitate in 6 N sodium hydroxide, add a few milligrams of carriers for gallium and germanium, and reprecipitate the silica with perchloric acid and leach with hydrochloric acid as described above.

Dissolve the precipitate in 6 to 8 drops of 6 N sodium hydroxide, and add 6 ml of 10 per cent. w/v ammonium molybdate solution and then sulphuric acid dropwise until the yellow molybdosilicate colour appears. Add 5 ml of 9 N sulphuric acid, and extract the molybdosilicate into 5 ml of pentanol. Discard the aqueous layer, and wash the organic layer successively with 5-ml portions of 9 N sulphuric acid and water. Back-extract the molybdosilicate into 2 ml of 10 per cent. w/v sodium acetate solution, and discard the organic layer.

Heat the solution with perchloric acid until fumes are evolved to precipitate silica and molybdenum trioxide. Dilute to 5 ml with water, and make alkaline with ammonia solution to dissolve molybdenum trioxide. Spin in a centrifuge, and discard the supernatant liquid. Wash the precipitated silica successively with 5-ml portions of ammonia solution, water and ethanol, and mount for counting and for determining the chemical yield.

COMPARATIVE STANDARDS—

A sample of a few milligrams of pure silicon was irradiated simultaneously with the gallium arsenide, to be used as a comparative standard. This was dissolved in hydrofluoric - nitric acid, and silica was precipitated with ammonium carbonate. The silica was dissolved in sodium hydroxide solution, and reprecipitated for counting and for determining the chemical yield by heating with perchloric acid. Although it is usual to have comparative standards of a few micrograms of the element in solution in a silica ampoule irradiated simultaneously with the solid sample to be investigated, this is not practical for silicon owing to the chemical nature of the ampoule and the instability of dilute solutions of silicon.

If 100 counts per minute is taken as the lowest limit of sensitivity, then for an irradiation of 2½ hours at 5 MW and a decay time of 2½ hours, the sensitivity of the method has been found to be $1{\cdot}3 \times 10^{-9}$ g of silicon.

DETERMINATION OF ZINC

A method has been developed for separating and purifying zinc from irradiated gallium arsenide. As with silicon, decontamination from arsenic was achieved by precipitation of basic zinc carbonate from an alkaline carbonate solution. Gallium was removed by dissolving the precipitate in hydrochloric acid and passing the solution through a column of anion-exchange resin. Basic zinc carbonate was reprecipitated and then redissolved, and zinc mercuri-thiocyanate was precipitated in the presence of oxalic acid. The precipitate was dissolved in the minimum amount of nitric acid, and the solution adjusted to pH 7 with ammonia solution. Zinc quinaldate was precipitated as the gravimetric compound for determining the chemical yield and for counting. The precipitate was washed with water and acetone, and mounted on a planchette. The complete radiochemical procedure, which is described below, took approximately 1 hour to complete.

PROCEDURE—

Transfer the irradiated gallium arsenide to a dissolution cell containing 1 ml of concentrated nitric acid and 10 mg of zinc carrier. Dissolve the sample by passing a current of 1 to 2 amps at 15 volts for 3 to 4 minutes.

Transfer the solution and one washing to a beaker, and add solid sodium carbonate until the solution is alkaline to methyl orange. Spin in a centrifuge, and discard the supernatant liquid. Wash the precipitate with water, spin in a centrifuge, and discard the supernatant liquid.

Dissolve the precipitate in the minimum amount of concentrated hydrochloric acid, make the solution 2 M in hydrochloric acid, and allow this solution to percolate through a column of anion-exchange resin (see Note 1). Wash the column with a further seven 2-ml portions of 2 M hydrochloric acid at a flow rate of 1 to 2 ml per minute.

Remove the zinc from the column with 6 ml of hot water containing a few milligrams of potassium iodide (see Note 2).

Add 1 mg each of gallium and arsenic carriers to this solution, and make alkaline with excess of sodium carbonate. Spin in a centrifuge, and discard the supernatant liquid.

Dissolve the precipitate in 2 ml of M nitric acid, and add 1 ml of saturated oxalic acid solution and 1 ml of mercuri-thiocyanate reagent (see Note 3). Stir for 2 to 3 minutes, then spin in a centrifuge, and discard the supernatant liquid.

Dissolve the zinc mercuri-thiocyanate in 0·2 ml of concentrated nitric acid (with care), and add 2 ml of water and chlorophenol red. Make the solution just alkaline with ammonia solution. Add glacial acetic acid until the solution is just acid, dilute to 8 ml, and add 1 ml of 3 per cent. w/v quinaldic acid. Heat in a water bath, then spin in a centrifuge, and discard the supernatant liquid. Wash the precipitate with water and acetone, and transfer to a planchette for weighing and counting.

NOTES—

1. The column was approximately 3 cm long and 0·7 cm in diameter. The resin used was Amberlite CG-400, which was washed with 2 M hydrochloric acid and water and kept in 2 M hydrochloric acid.

2. The exchange time Zn^{2+} (column) → Zn^{2+} (aqueous) is relatively long, so at least 10 minutes should be allowed for this step.

3. Mercuri-thiocyanate reagent was prepared by dissolving 27 g of mercuric chloride and 39 g of ammonium thiocyanate in 1 litre of water.

INTERFERENCES—

Sources of zinc quinaldate produced from irradiated gallium arsenide by this procedure were found to contain considerable zinc activities that have been identified as arising from the reactions of fast neutrons on gallium, *viz.*—

$$^{69}\mathrm{Ga}\ (n,p)\ ^{69}\mathrm{Z}\bar{\mathrm{n}} \xrightarrow[\mathrm{I.T.}]{14\ \mathrm{h}} {}^{69}\mathrm{Zn} \xrightarrow[\beta]{52\ \mathrm{m}} {}^{69}\mathrm{Ga}\ \text{(stable)}$$

$$^{69}\mathrm{Ga}\ (n,p)\ ^{69}\mathrm{Zn} \xrightarrow[\beta]{52\ \mathrm{m}} {}^{69}\mathrm{Ga}\ \text{(stable)}.$$

That the 14-hour activity on the sources was not due to the presence of ^{72}Ga was demonstrated by the facts that no proportional 20-minute ^{70}Ga was detected and that the 14-hour activity did not show a peak at 0·84 MeV in its gamma-ray spectrum. Thus the zinc activities produced by the reaction of fast neutrons with gallium will interfere in the determination of zinc in gallium arsenide.

A measurement of the magnitude of the interference has been carried out. Samples of zinc and gallium were irradiated in the reflector region of Merlin at 100 kW, the neutron fluxes at this power being $1{\cdot}5 \times 10^{12}$ thermal neutrons per sq. cm per second and $2{\cdot}1 \times 10^{11}$

fast (>1 MeV) neutrons per sq. cm per second. Radiochemically pure zinc was separated from each sample, and the decay curves were resolved. The ratio obtained was—

$$\frac{^{69}\text{Zn, c.p.m. per g of gallium}}{^{69}\text{Zn, c.p.m. per } \mu\text{g of zinc}} = \frac{7{\cdot}9 \times 10^6}{1{\cdot}5 \times 10^4}$$

Thus 0·5 g of gallium, which is approximately equivalent to 1·0 g of gallium arsenide, will given an apparent zinc content of 265 μg.

The magnitude of this interference may be considerably reduced by irradiating samples in the thermal column where, at a reactor power of 5 MW the fluxes are $2{\cdot}5 \times 10^{11}$ thermal neutrons per sq. cm per second and $2{\cdot}4 \times 10^8$ fast neutrons per sq. cm per second. At these fluxes, the ^{69}Zn produced by each method would be $2{\cdot}5 \times 10^3$ c.p.m. per μg of zinc from ^{68}Zn (n,γ) ^{69}Zn and $9{\cdot}0 \times 10^3$ c.p.m. per g of gallium from ^{69}Ga (n,p) ^{69}Zn. This gives an apparent zinc impurity of 1·8 μg per g of gallium arsenide. The contribution from the fast-neutron reaction can be determined by simultaneously irradiating samples with and without cadmium covers. The use of a cadmium cover reduces the slow-neutron reaction ^{68}Zn (n,γ) ^{69}Zn by a considerable amount, and then by comparing the amounts of ^{69}Zn produced in the covered and uncovered samples the amount of zinc present may be inferred by difference. If this method is used in the thermal column, a zinc impurity of 0·1 μg per g of gallium arsenide would give a 20 per cent. difference between cadmium-covered and uncovered samples.

DETERMINATION OF MAGNESIUM

The main activity produced during a short irradiation of pure gallium arsenide has a half-life similar to that of ^{27}Mg (^{70}Ga, $t_{\frac{1}{2}} = 21$ minutes). A rapid separation procedure that removed all gallium activities without complete removal of the long-lived arsenic activity may have been acceptable in the interest of sensitivity, but it has been found possible to remove all gallium and arsenic activities in a procedure taking 20 minutes.

After dissolution of irradiated gallium arsenide and equilibration with magnesium carrier, magnesium (and some gallium) was precipitated on addition of sodium hydroxide. The precipitate was dissolved in hydrochloric acid, and the solution was passed through a column of anion-exchange resin, on which the gallium was adsorbed. Magnesium hydroxide was precipitated from the effluent and then redissolved, and ammonium magnesium phosphate was precipitated from a citric acid solution and used for preparing sources and determining the chemical yield. The complete procedure is described below.

PROCEDURE—

Transfer the irradiated gallium arsenide to a dissolution cell containing 1 ml of concentrated nitric acid, 10 mg of magnesium carrier and a few milligrams of germanium and zinc carriers. Dissolve the sample by passing a current of 1 to 2 amps at 15 volts for 3 to 4 minutes.

Transfer the solution to a beaker, and add excess of solid sodium hydroxide. Boil for 1 minute, transfer to a centrifuge tube with washings of water, spin in a centrifuge, and discard the supernatant liquid. Wash the precipitate thoroughly with water, and discard the washings.

Dissolve the precipitate in 1 ml of concentrated hydrochloric acid, and adjust to 6 N in hydrochloric acid. Pass the solution through a column of anion-exchange resin (see Note 1) at a flow rate of 1 to 2 ml per minute, and then pass 2 ml of 6 N hydrochloric acid through the column (see Note 2). Collect the effluent in a centrifuge tube.

Add gallium and arsenic carriers to the solution, and precipitate magnesium hydroxide with solid sodium hydroxide as described above. Spin in a centrifuge, discard the supernatant liquid, wash the precipitate with water, and discard the washings.

Dissolve the precipitate in concentrated hydrochloric acid, and dilute to 10 ml. Add 0·1 g of citric acid, 2 or 3 drops of 10 per cent. w/v potassium iodide solution and 1 ml of 20 per cent. w/v ammonium dihydrogen orthophosphate solution, and make the solution alkaline by adding ammonia solution to precipitate ammonium magnesium phosphate. Spin in a centrifuge, and discard the supernatant liquid.

Dissolve the precipitate in concentrated hydrochloric acid, and reprecipitate with ammonia solution as described above. Wash the precipitate successively with 10 per cent. v/v ammonia solution, water and ethanol, and mount for counting and determining the chemical yield.

NOTES—

1. The column was approximately 2 cm long and 0·7 cm in diameter. The resin used was Amberlite CG-400 equilibrated with 6 N hydrochloric acid.

2. Excessive washing of the column has been found to introduce gallium into the effluent, and should therefore be avoided.

SENSITIVITY—

If 100 counts per minute is taken as the lowest limit of sensitivity, then for an irradiation of 10 minutes at 5 MW and a decay time of 20 minutes, the sensitivity of the determination is $7{\cdot}1 \times 10^{-8}$ g of magnesium.

THERMAL NEUTRON SELF-SHIELDING IN GALLIUM ARSENIDE

The magnitude of thermal neutron self-shielding is determined by the extent of overlapping of neutron resonances in the host and trace materials. When this effect is expected, its magnitude must be investigated and, when necessary, suitable corrections applied to the results.

Okada[7] gives an empirical formula for the self-shielding effect, and application of this formula to a weight of 1 g of gallium arsenide suggests that the self-shielding should be less than 10 per cent.

The effect of self-shielding on the determination of silicon and magnesium in gallium arsenide has been investigated, the irradiations being carried out in the Merlin reactor. The effect of self-shielding on the determination of zinc has not been attempted, as the interference of fast-neutron reactions is considerable in this determination.

EXPERIMENTAL—

Small pieces of the element under investigation were irradiated with and without a gallium arsenide cover. The cover consisted of a cube of gallium arsenide with a 6-mm side and a cylindrical hole in the centre of diameter 2 mm and 4 mm deep. The top of the cube was covered by a 2-mm slice of gallium arsenide. A gallium arsenide covered specimen and an uncovered specimen were held in known positions relative to each other in a Perspex jig in an irradiation can. After irradiation the two samples were processed to bring them to a form suitable for weighing and counting. Each sample was checked for purity by measurement of half-life. A second irradiation was carried out with the same sample jig, but without the gallium arsenide cover. Each sample was processed in an identical way to the first pair, weighed, and counted. The difference in the specific activity of the latter pair represented the correction to be applied to take account of flux differences between the two positions.

RESULTS—

Silicon—Two types of irradiation facility were used for this experiment; the blanking-element facility and the fast-transfer facility. The activities induced in samples of silicon covered with gallium arsenide and uncovered samples, corrected for flux variations as described above, are shown in Table I.

TABLE I

ACTIVITIES INDUCED IN SILICON WITH AND WITHOUT GALLIUM ARSENIDE COVER

Irradiation facility		Activity, c.p.m. per mg of Si*
Blanking element	Uncovered	$8{\cdot}95 \times 10^3$
	Covered	$8{\cdot}94 \times 10^3$
Fast transfer	Uncovered	$5{\cdot}45 \times 10^4$
	Covered	$5{\cdot}55 \times 10^4$

* For irradiations of 2 hours at a reactor power of 10 kW.

It is apparent that neutron self-shielding will not cause serious errors in the determination of silicon in gallium arsenide by thermal-neutron activation analysis.

Magnesium—Similar experiments have been carried out for magnesium; irradiation was for a short time in the fast-transfer facility. The results obtained, corrected for flux variation, are shown in Table II.

TABLE II

ACTIVITIES INDUCED IN MAGNESIUM WITH AND WITHOUT GALLIUM ARSENIDE COVER

	Uncovered	Covered	Uncovered	Covered
Activity, c.p.m. per mg of Mg*	$6{\cdot}20 \times 10^3$	$6{\cdot}25 \times 10^3$	$6{\cdot}34 \times 10^3$	$6{\cdot}5 \times 10^3$

* For irradiations of 10 minutes at a reactor power of 10 kW.

Again it is apparent that neutron self-shielding will not lead to serious error in the determination of magnesium in gallium arsenide.

CONCLUSIONS

Methods have been developed for determining silicon, zinc and magnesium in gallium arsenide, with the separation of short-lived species. The sensitivities for silicon and magnesium are $1{\cdot}3 \times 10^{-9}$ g and $7{\cdot}1 \times 10^{-8}$ g, respectively. For zinc, a fast-neutron reaction with gallium interferes, but 1×10^{-7} g can be determined under specified conditions. The sensitivities for silicon and magnesium represent the limits of the method, but for a host material that does not interfere in the detection of zinc, $2{\cdot}5 \times 10^{-10}$ g of zinc could be detected.

The effect of thermal neutron self-shielding by gallium arsenide in the detection of silicon and magnesium has been shown to be insignificant. Since the magnitude of this effect is dependent on the position of neutron absorption resonances in the host and trace constituents, this result is not directly applicable to the determination of other trace materials or other matrixes.

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Determination of Zirconium-95 and Niobium-95 in Seaweed and Sea Water

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A method is described for determining zirconium-95 and niobium-95 in seaweed and sea water.

Oxalic acid in nitric acid is used to complex both elements, and, after destruction of the oxalic acid with potassium chlorate, zirconium is precipitated as zirconium phosphate and niobium as niobic acid. After removal of rare-earth activities, zirconium is separated as barium fluorozirconate, decontaminated from other active isotopes, and finally ignited to zirconium dioxide; the niobium fraction is similarly decontaminated, and finally ignited to niobium pentoxide. The oxides are then determined by gamma-ray spectrometry.

METHODS for measuring radionuclides in sea-water and marine organisms, including marine foodstuffs, are essential in the control of the discharge of radioactive effluent into the sea. Safe limits for the release of radioactivity from a nuclear installation are calculated from the results of preliminary studies on radionuclides discharged at low release rates, and are then checked by regular monitoring during normal operation. An assay procedure is described here for zirconium-95 and niobium-95 in environmental materials. Both these nuclides are among the more important fission products in the effluent discharged from plant processing spent nuclear fuel.

The two nuclides are normally present together in effluent, but the proportions released vary, and individual measurement is necessary since the maximum permissible concentrations in drinking water[1] and in foodstuffs, which set the release limits, are different. Separate measurement is essential in the investigation of the distribution patterns of the two elements between sea water, biota and sediment. Further, correction for radioactive decay during handling of samples is facilitated by simultaneously determining both isotopes, as the transient parent - daugher relationship can then be resolved.

Zirconium-95 and niobium-95 can be measured together by gamma-ray spectrometry, but cannot be readily distinguished because of close energy levels. Difficulty was experienced in applying the di-n-butyl phosphoric acid separation proposed by Scadden and Ballou[2] to biological ash, owing to extraction of large amounts of inorganic constituents. A modification of Kahn's phenylarsonic acid procedure[3] gave only 20 to 30 per cent. recovery of zirconium when applied to material having a high phosphate content. Separation of these nuclides by precipitation of the zirconium as barium fluorozirconate, as used by Hume,[4] was adopted after they had been obtained from the sample by precipitation with a carrier as zirconium phosphate and niobic acid. Subsequently, a series of radiochemical purification steps was carried out, each designed to give the required degree of decontamination from particular groups of active or inactive elements present or expected to be present. Radio-assay was then carried out by gamma scintillation counting.

METHOD

REAGENTS-

Zirconium carrier solution, 10 *mg per ml*—Dissolve 7·5 g of high purity zirconium nitrate (obtainable from Johnson, Matthey & Co. Ltd.) in 250 ml of water. Add a few drops of 16 M nitric acid. Standardise the solution by evaporating 5-ml portions to dryness in silica crucibles and igniting at 800° C for 3 hours; weigh the residue as zirconium dioxide.

Niobium carrier solution, 10 *mg per ml*—Dissolve 6·5 g of potassium hexaniobate in about 50 ml of water. Add, slowly with stirring, 4 ml of 16 M nitric acid to the near-boiling solution, and then heat, and stir for 5 minutes. Filter, and wash the precipitated niobic acid with a hot 2 per cent. solution of ammonium nitrate. Dissolve the washed precipitate in 50 ml of a hot saturated solution of oxalic acid, filter, dilute the filtrate to 250 ml, and store in a polythene container. To standardise the solution boil 5-ml portions with 30 ml of 6 M nitric acid and 1 g of potassium chlorate. Cool, and add 16 M ammonium hydroxide until the pH of the solution is 8 to 10. Collect the precipitate on a Millipore HA membrane filter, and wash with hot water. Ignite at 800° C for 3 hours, and weigh the residue as niobium pentoxide.

Standard zirconium-95 - niobium-95 solution, approximately 0·02 *μC per ml*—An old, *i.e.*, near equilibrium, isotopic mixture standardised with respect to both isotopes is obtainable from the Radiochemical Centre, Amersham; it should be used only up to 6 months from the time of standardisation.

Lanthanum carrier solution, 10 *mg per ml*—Dissolve 3·2 g of lanthanum nitrate in 100 ml of water.

Ruthenium carrier solution, 10 *mg per ml*—Dissolve 6·3 g of ruthenium trichloride in 250 ml of 0·1 M hydrochloric acid.

Cupferron solution—Dissolve 6 g of analytical-reagent grade cupferron (having a sulphated ash content <0·01 per cent.) in 100 ml of water. Store the solution in a refrigerator.

Thioacetamide solution, 100 *mg per ml.*

Mandelic acid solution, saturated.

Thioglycollic acid, 20 *per cent. v/v.*

APPARATUS—

Gamma counting equipment—A scintillation counter having a sodium iodide - thallium well crystal protected with steel and lead, and a single-channel pulse-height analyser.

Glassware—Treated with dimethyldichlorosilane.

PROCEDURE—

Preliminary isolation from seaweed—Carefully wash the seaweed with sea water during collection. Ash the seaweed quantitatively by drying at 140° C, and ignite at 500° C in silica; then heat at 800° C in platinum to remove carbon, and grind the residue to pass a 240-mesh sieve. Add 20 ml of M nitric acid - 0·02 M oxalic acid per g of ash, and heat, with stirring, to 70° C. Add 1·00 ml each of niobium and zirconium carrier solutions, and stir; then add 0·25 g of potassium chlorate per 20 ml of acid, and boil gently to precipitate zirconium phosphate and niobic acid. Cool, and remove the precipitate by spinning in a centrifuge; repeat with a further 1·00 ml of both carrier solutions. Combine the precipitates, and wash with 20 ml of 3 M hydrochloric acid. Digest the washed precipitate with 5 ml of 40 per cent. hydrofluoric acid for 1 hour at 100° C in a capped polypropylene tube. Spin in a centrifuge, and repeat the digestion on any residue. Transfer the solution to a platinum dish, and evaporate to dryness with 20 ml of 16 M nitric acid. Carry out a second evaporation with further portions of hydrofluoric and nitric acids, dissolve the residue in 2 ml of 40 per cent. hydrofluoric acid, transfer to a nitrocellulose centrifuge tube, and dilute to 10 ml.

Preliminary isolation from sea water—Collect filtered sea water in a polythene bottle. Measure the volume, and transfer the entire sample to a beaker, washing the bottle with 20 ml of 0·5 per cent. oxalic acid per litre of sea water. Add 1·00 ml each of niobium and zirconium carrier solutions, and then, for each litre of sea water, 100 ml of 16 M nitric acid, 1 ml of phosphoric acid, sp.gr. 1·75, and 1 g of potassium chlorate. Extract zirconium-95 and niobium-95 by heating to 90° C until a precipitate forms and coagulates sufficiently for complete collection by decanting and spinning in a centrifuge. Add a further 1·00 ml of each carrier solution, and repeat the scavenge. Combine the two precipitates, and transfer to a polypropylene centrifuge tube with the aid of 20 ml of 3 M hydrochloric acid. Dissolve the precipitate in 2 ml of 40 per cent. hydrofluoric acid, and heat in a boiling-water bath to ensure equilibration of the active isotopes with the carriers. Dilute to 10 ml, and transfer to a nitrocellulose centrifuge tube.

Separation, purification and assay of zirconium-95 and niobium-95—Remove rare-earth activities by a double lanthanum fluoride scavenge, with 0·5 ml of lanthanum carrier solution each time. Separate zirconium by precipitation with 2 ml of saturated barium nitrate solution. Suspend the barium fluorozirconate in 2 ml of saturated boric acid solution, and redissolve in 1 ml of 16 M nitric acid and 10 ml of water, neglecting any insoluble barium sulphate; reprecipitate with 1 ml of 40 per cent. hydrofluoric acid. Combine the two solutions for the niobium fraction.

For adequate decontamination of the zirconium fraction from radioactive niobium, carry out a third precipitation cycle; then dissolve the precipitate in 3 ml of 11 M hydrochloric acid, 1 ml of saturated boric acid solution and 26 ml of water, and precipitate the barium with 2 drops of sulphuric acid, sp.gr. 1·84. Scavenge active ruthenium isotopes by adding 1 ml of ruthenium carrier solution and 1 ml of thioacetamide solution, and digesting at 90° C until coagulation is complete and the solution is colourless. Filter through a Millipore HA membrane filter. Finally, recover the zirconium by precipitation with 18 M ammonium hydroxide in the presence of 2 ml of 20 per cent. thioglycollic acid solution added to complex the iron; wash the precipitate with a 2 per cent. solution of ammonium nitrate. Dissolve the washed precipitate in 10 ml of 6 M hydrochloric acid, and reprecipitate with 10 ml of saturated mandelic acid solution by heating in a boiling-water bath. Collect the precipitate on a filter-paper, wash with boiling water, and ignite at 800° C for 3 hours. Weigh the zirconium dioxide into a gamma counting tube, count, and calculate the recovery of zirconium.

Decontaminate the niobium fraction from radioactive zirconium by adding 1 ml of zirconium carrier solution and precipitating with 1 ml of saturated barium nitrate solution. Remove ruthenium isotopes as described above; use a polytetrafluoroethylene or polypropylene beaker or flask. To recover niobium from the hydrofluoric acid medium, mix the solution with 35 ml of saturated boric acid solution and 10 ml of 11 M hydrochloric acid. Extract the cupferron complex into chloroform by using 4 ml of 6 per cent. cupferron solution and 20 ml of chloroform for a first 1-minute extraction and 2 ml of cupferron solution and 10 ml of chloroform for second and third 1-minute extractions. Cool all solutions to 10° C. Destroy organic matter by heating the chloroform phase with 3 ml of sulphuric acid, sp.gr. 1·84, and 20 ml of 16 M nitric acid until the solution is nearly colourless and about 3 ml in volume. Add 1 or 2 drops of 16 M nitric acid to the boiling solution to complete the oxidation. Precipitate niobium in the presence of 2 ml of 20 per cent. thioglycollic acid by adding 16 M ammonium hydroxide until the pH is 10, and wash the precipitate twice by boiling with 20 ml of 1·2 M ammonium hydroxide - 0·6 M ammonium nitrate solution, separating the precipitate each time by spinning in a centrifuge. Immediately, dissolve the washed precipitate in 0·5 ml of 6 M nitric acid and 2 ml of saturated oxalic acid solution, and reprecipitate the niobium by heating with 10 ml of 16 M nitric acid and 0·5 g of potassium chlorate; digest in a boiling-water bath for 15 minutes. Separate the precipitate by spinning in a centrifuge, and suspend in 30 ml of 0·6 M nitric acid - 1·2 M ammonium nitrate solution. Boil, filter through a Millipore HA membrane filter, and wash the filter with 2 to 5 ml of the acidic wash solution. Ignite the precipitate at 800° C for 3 hours. Weigh the niobium pentoxide into a gamma-counting tube, count, and calculate the recovery of niobium.

For radio-assay mount the sources in glass tubes having wall thickness at least 200 mg per sq. cm to eliminate the beta radiation. Count the gamma radiation from zirconium-95 and niobium-95 through a 0·72- to 0·76-MeV window selected by setting up on a source containing the two isotopes in similar proportions. Calibrate the counter with a standard zirconium-95 - niobium-95 source at a counting rate within the linear response region of the instrument.

CALCULATION—

The activity of the standard source, A_c, in picocuries (pC) at the time of counting is given by—

$$A_c = Z_0 \left[e^{-\lambda_1 t} + \frac{\lambda_2}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}) \right] + N_0 e^{-\lambda_2 t}$$

where Z_0 = the activity due to zirconium-95 at the time of standardisation (pC),
N_0 = the activity due to niobium-95 at the time of standardisation (pC),
t = the interval between standardisation and counting,
λ_1 = the disintegration constant for zirconium-95 and
λ_2 = the disintegration constant for niobium-95.

The zirconium-95 activity at the time of sampling, Z_S, is given by—

$$Z_S = Z_C\, e^{\lambda_1 T}$$

and the niobium-95 activity at the time of sampling, N_S, is given by—

$$N_S = N_C\, e^{\lambda_2 T} - Z_S \frac{\lambda_2}{\lambda_2 - \lambda_1} \left[e^{(\lambda_2 - \lambda_1)T} - 1\right]$$

where Z_C = the zirconium-95 activity at the time of counting,
N_C = the niobium-95 activity at the time of counting and
T = the interval between sampling and counting.

DISCUSSION OF THE METHOD

The problem of collecting the two isotopes from bulky samples of natural materials is most easily solved by sample dissolution and carrier exchange in nitric acid containing oxalic acid as complexing agent for both elements. Subsequent destruction of the oxalic acid with chloric acid in the same acidic medium leads to the precipitation of zirconium as phosphate and niobium as niobic acid, with little occlusion of matrix constituents, and at the same time considerable decontamination from most other active isotopes. This self-scavenging procedure is readily applicable to a wide range of samples. Hydrofluoric acid is undoubtedly a better complexing agent for both elements, but their recovery in isolation is more difficult, and the use of this reagent in large scale operations is to be avoided.

When seaweed is analysed as a foodstuff, complete dissolution of the sample, including siliceous material, is required, as all may be ingested by man. Matter insoluble in nitric - oxalic acid mixture often amounts to about 1 per cent. of the ash. Dissolution of this component can be achieved either by fusion with potassium hydrogen sulphate and then extraction with oxalic acid or by digestion with hydrofluoric acid. Both gave about 98 per cent. recovery of zirconium-95 - niobium-95 from the ash of seaweed and detritus samples taken from the shore. After this treatment removal of silica, at least down to 20 mg, is essential. After digestion with hydrofluoric acid, this can be accomplished by evaporation with nitric acid. Each stage of such an evaporation gives a ten-fold reduction in silica with negligible loss of volatile niobium pentafluoride, which would occur if sulphuric acid was used.

When seaweed is analysed as a biological entity, most of the siliceous matter may be considered extraneous; such matter, if finely divided, may adsorb considerable amounts of zirconium-95 and niobium-95 from sea water. However, such directly adsorbed activity will be rendered refractory during ashing, and more relevant results may be obtained by rejecting the matter insoluble in nitric - oxalic acid mixture. Zirconium-95 - niobium-95 activity associated with the soluble salts in seaweed ash dissolves readily in this medium, as was shown by a trial experiment in which 98·7 per cent. of zirconium-95 - niobium-95 mixture, added to the ash immediately before the final treatment at 800° C, was recovered.

Geiger[6] has shown that ruthenium can be precipitated by thioacetamide from 2 M hydrochloric acid containing 0·08 M hydrofluoric acid. Experiments carried out in connection with this procedure indicated that the operation can also be conducted in 2 M hydrofluoric acid. Mean decontamination factors from ruthenium-106 for the entire procedure were 1×10^5 for zirconium fractions and 5×10^4 for niobium fractions. These are adequate for monitoring samples in which ruthenium-106 predominates, *e.g.*, current samples from the Windscale area, the bias from this cause not exceeding +1 per cent.

The method has so far been applied to the edible seaweed Porphyra used in the manufacture of laverbread, to the more widely occurring Fucus species of seaweed often chosen in fallout and effluent dispersal studies, to fish flesh and to sea water. Chemical recovery is normally about 70 per cent. for zirconium and 80 per cent. for niobium. A test of the procedure with zirconium-95 - niobium-95 added to seaweed ash indicated a coefficient of variation of 2 per cent. (5 replicate determinations), with a bias of −2 per cent. Similar analytical variation was found for both isotopes on environmentally contaminated samples of seaweed.

The sensitivity attainable depends on sample size and counting statistics. Gamma counting was adopted for routine measurements because of easy source preparation. When 20 g of seaweed ash or 5 litres of sea water were used, 0·2 pC of zirconium-95 or niobium-95 per g of seaweed or 0·02 pC per g of sea water could be determined with an over-all coefficient of variation of 3 per cent. by the gamma-counting method described. With these amounts, measurements of this accuracy can be made down to 10^{-4} times the maximum permissible concentration in seaweed; for measurements at this level two sources and background can be counted in 24 hours if equipment having a counting efficiency of 9 per cent. and a background rate of 12 counts per minute is used. A ten-fold increase in sensitivity can be obtained by low-level soft beta-particle counting,[5] although this involves the spreading of uniformly thin sources and manipulation of gas-flow counters. However, this extra sensitivity is needed for accurate measurements of zirconium-95 and niobium-95 at the low concentrations found in fish flesh arising from fallout.

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A Sensitive Microbiological Assay Procedure for determining Magnesium in Biological Materials

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A simple microbiological assay procedure for determining magnesium in the range 1 to 8 μg has been developed, in which *Neurospora crassa* Em 5297a is used as the test organism. For the range 1 to 4 μg of magnesium, an indirect assay procedure based on the determination of the mycelial content of the "phenol reagent - positive material" has also been evolved, the dry weight of mycelia being directly used as an index of response for higher levels of magnesium. The method has been found to be applicable to various biological tissues, the recoveries being about 95 to 105 per cent.

ATTEMPTS to develop microbiological methods for determining metals have so far been mainly unsuccessful. Manganese has been assayed with *Lactobacillus arabinosus* 17–5 in biological materials,[1] and *Aspergillus niger* has been used for determining several metals, including magnesium.[2] However, the *A. niger* procedure for determining magnesium requires a sample containing at least 50 μg of metal at a concentration of 1 μg per ml of culture medium. Macleod and Snell[3] studied the magnesium requirements of several *Lactobacilli* with a view to using them for the assay of magnesium, but found that, even with pre-treatment of a magnesium deficient medium with *Lactobacilli*, it was not possible to reduce growth in an unsupplemented medium. During earlier work with the mould *Neurospora crassa*[4] it was found that the wild strain of this mould, Em 5297a, had a low requirement for magnesium, and growth was totally absent if pure salts were used to constitute the medium and magnesium was omitted. Advantage was taken of this observation, and a sensitive microbiological assay procedure is described here, in which this mould is used as the test organism.

METHOD

APPARATUS—

The mould was grown in Pyrex-glass test-tubes (150 mm × 15 mm) on a reciprocal shaker (stroke 10·8 cm; 84 cycles per minute) in a constant-temperature chamber kept at 30° ± 1° C; the test-tubes were kept inside the chamber in a test-tube rack mounted so that the tubes were tilted, lips upwards, so as to spread the medium over a length of about 7 to 8 cm. After inoculation, the organism was grown with continuous shaking during the entire growth period.

Pyrex glassware was used throughout; it was cleaned with chromic - sulphuric acid, and then rinsed with distilled water, and, finally, with water distilled in an all-glass apparatus.

REAGENTS—

Acids—Analytical-reagent grade acids were used; the concentrated nitric acid was further purified by distillation before use.

Folin and Ciocalteu's phenol reagent—Prepared as recommended by Lowry, Rosebrough, Farr and Randall.[5]

ASSAY MEDIUM AND GROWTH TECHNIQUE—

The culture medium used in all assays was based on that used in earlier work[4] with the difference that magnesium sulphate was replaced by sodium sulphate to provide an equivalent amount of sulphur. The percentage composition was: glucose, 2; potassium dihydrogen orthophosphate, 0·3; ammonium nitrate, 0·2; ammonium tartrate, 0·1; sodium sulphate, 0·025; sodium chloride, 0·01; calcium chloride, 0·01.

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The trace elements supplied (in μg per 100 ml) were: zinc, 20; manganese, 20; copper, 8; iron, 2; molybdenum, 2. Biotin (0·5 μg per 100 ml) was added, and one drop of Tween 80 per 100 ml of medium was also added to inhibit sporulation.[6]

For assay purposes, the organism was grown on the above medium (adjusted to pH 4·8 to 5·0) for 72 hours at 30° ± 1° C with shaking as described above. A 2·0-ml portion of double-strength medium and 2·0 ml of sample in aqueous solution adjusted to the pH of the medium were taken in each assay tube.

STOCK CULTURE AND INOCULUM—

Neurospora crassa Em 5297a was used as the assay organism. It was maintained by transfer biweekly on agar slants of the above medium supplemented with 0·2 per cent. each of yeast and malt extracts and containing 2 per cent. of agar. For inoculation, spore from a 7-day-old culture was used, one drop of a suspension of spore having a transmission of approximately 90 per cent. in sterile water distilled in an all-glass apparatus being used in each tube.

ASSESSMENT OF GROWTH REPONSE—

At the end of the growth period, the mycelia were carefully removed, washed, and dried at 60° C overnight; the assay was then completed by determining the mycelial weight.

In assaying magnesium in the range 1 to 4 μg, the mycelia were carefully removed, rinsed on a filter-paper with water distilled in an all-glass apparatus, and transferred to Pyrex-glass test-tubes (100 mm × 10 mm). A 0·5-ml portion of 5 N sodium hydroxide was added to the contents of each tube, and the tubes were heated on a boiling-water bath for 30 minutes; they were then cooled, and the contents were adjusted to 5 ml with distilled water. Portions (0·5 ml) were removed, after centrifugation if necessary, for determining the "phenol reagent - positive material" according to the method of Lowry *et al.*,[5] a Klett-Summerson photo-electric colorimeter and a No. 66 red filter being used.

PREPARATION OF SAMPLE—

The biological sample was wet-ashed in a 50-ml Pyrex-glass conical flask with 10 ml of concentrated nitric acid and 1·0 ml of 66 per cent. perchloric acid (Merck G.R.) per gram of the sample on a sand-bath. After complete evaporation, the digest was treated with 5 ml of concentrated hydrochloric acid, and once again evaporated to dryness. To the residue dissolved in 5·0 ml of distilled water was added 0·1 ml of a 50 per cent. w/v solution of ammonium chloride, and then 0·05 ml of ammonia solution, sp.gr. 0·88, was added to make the solution alkaline. A 0·1-ml portion of a 0·5 per cent. solution of ferrous sulphate was added and hydrogen sulphide was bubbled through the solution for 15 to 20 minutes. After the solution had been set aside for 2 hours, the precipitated ferrous sulphide, together with sulphides of other heavy metals, if any, was removed by filtration through a fluted Whatman No. 42 filter-paper, and washed with 15 to 20 ml of distilled water. The filtrate and the washings were combined, and evaporated to dryness on a sand-bath, 1·0 ml of concentrated nitric acid being added during the later stages of the evaporation. The residue was dissolved in a known volume of distilled water, and adjusted to pH 4·8 to 5·0. Portions of this solution were used for the assay.

RESULTS

RANGE OF ASSAY—

Under the conditions of growth obtaining in the proposed procedure, when analytical-grade reagents are used and normal precautions are taken to eliminate magnesium as a contaminant, growth is negligible on the basal medium constituted without magnesium. The microbiological response curve obtained is shown in Fig. 1.

It will be seen that the useful range of assay is from 1 to 8 μg of magnesium. The mycelial weights have been found to be highly reproducible, variation between duplicates never exceeding 0·2 mg over the entire range.

For samples containing 1 to 4 μg of magnesium, the indirect method of estimating growth based on "phenol reagent - positive material" has been developed to ensure higher accuracy without involving elaborate manipulation. If the mycelia are pressed gently to dryness before suspension in sodium hydroxide, the alkali digest can be directly adjusted to volume by adding 4·5 ml of water, and portions can be removed for assay. Under the conditions proposed, we have found the extraction of "phenol reagent - positive material" is complete in one step. In the range 1 to 4 μg of magnesium, the "phenol reagent - positive material" gives a linear standard graph. Preliminary experiments showed that over 70 per cent. of the "phenol reagent - positive material" is protein. For samples containing less than 1·0 μg of magnesium, the microbiological assay procedure is not recommended, even with the indirect method based on "phenol reagent - positive material" of the mycelium.

TABLE I

APPLICABILITY OF THE *N. crassa* ASSAY PROCEDURE FOR DETERMINING MAGNESIUM IN BIOLOGICAL TISSUES

Sample	Portion taken for assay, ml	Magnesium found, μg	Total magnesium content, μg per g fresh weight	Magnesium recovered,* μg	Magnesium recovered, %
Tecoma stans leaves	0·2	2·50	625·0	3·50	100
	0·4	4·95	618·7	5·90	95
	0·5	6·20	620·0	7·10	90
L. sativus seeds ..	0·2	3·00	1500	3·95	95
	0·3	4·35	1450	5·30	95
	0·4	5·90	1475	6·90	100
Rat kidney ..	0·2	2·25	162·5	3·20	95
	0·4	4·60	166·1	5·55	95
	0·6	6·70	158·9	7·80	110
Rat blood	1·0	1·20	30·0†	2·20	100
	1·5	1·80	30·0†	2·75	95
	1·9	2·45	32·2†	3·40	95
Rat spleen.. ..	0·2	1·65	221·7	2·70	105
	0·4	3·40	228·5	4·40	100
	0·6	5·00	224·0	6·00	100
Rat liver	0·2	2·10	145·8	3·15	105
	0·4	4·30	149·3	5·25	95
	0·6	7·00	161·7	7·95	95

* Amount recovered after 1 μg of magnesium had been added.
† Expressed as μg per ml.

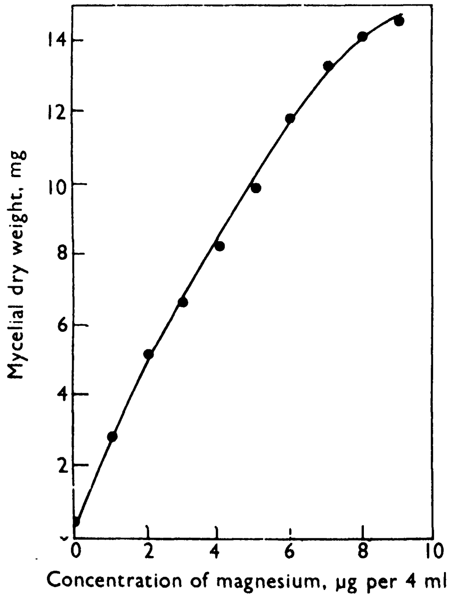

Fig. 1. Microbiological assay curve for magnesium with *N. crassa*

APPLICABILITY OF THE METHOD TO BIOLOGICAL TISSUES

The applicability of the proposed method has been studied for several biological tissues. Recoveries have been tested by adding magnesium at the 1·0-μg level to three different portions of each biological tissue tested. The absolute values and the mean recoveries obtained are shown in Table I, and represent values derived from at least four independent analyses for each type of sample. It can be seen that the method is suitable for the type of samples investigated throughout the concentration range.

INTERFERENCE FROM OTHER METALS

The effect of other metals on the assay of magnesium by the proposed procedure has been studied. The metals mentioned below do not interfere in the assay up to the levels indicated (μg per tube): sodium, 3000; potassium, 51,000; calcium, 2100; iron, 1000: zinc, 10; cobalt, 10; nickel, 5.

DISCUSSION OF THE METHOD

The only useful microbiological method for determining magnesium so far has been that of Nicholas[2] in which *A. niger* is used However, it involves the use of carefully purified media and a sample having a magnesium content of at least 50 μg. The considerably lower requirement for magnesium of *N. crassa* and the fact that elaborate purification of the assay medium is unnecessary are distinct advantages in the proposed method. Further, by use of a simple procedure for the indirect determination of growth reponse, it is possible to work conveniently with good precision at levels as low as 1 μg. The proposed procedure is also specific for magnesium, since calcium, sodium and potassium do not interfere, except at extremely high levels compared with the amount of magnesium present. Heavy metals, such as cobalt, nickel and zinc, which interfere with magnesium metabolism in the mould[4] and are often present in biological material in various amounts, were found to be completely eliminated by the hydrogen sulphide treatment introduced during the preparation of the sample. Since uniformly good recoveries were obtained for all the sample sizes with the various types of biological materials, the proposed procedure can be adopted with advantage, particularly since the conventional complexometric titration of magnesium[7] involves a correction for the presence of calcium and is of limited usefulness below 10 μg of magnesium.

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Pyrethrum Analysis: An Infrared Method

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A method of pyrethrum analysis is described, in which "pyrethrin I" is determined by infrared measurement. The results are similar to those obtained by the Association of Official Agricultural Chemists' method, and it is shown that the Pyrethrum Board of Kenya's method gives falsely high results for "pyrethrin I." The determination of "pyrethrin II" and the accuracy of pyrethrum analyses are discussed.

MANY methods have been proposed for the assay of pyrethrum, but in recent years commercial analyses have generally been conducted by one of two variants of the "mercury reduction" method originally devised by Wilcoxon[1] and modified by Holaday.[2] One version of the method, recommended by the Association of Official Agricultural Chemists,[3] is generally known as the A.O.A.C. method. The other (mainly used in the United Kingdom) is described in the British Pharmaceutical Codex,[4] the British Veterinary Codex,[5] and in leaflets published by the Pyrethrum Board of Kenya (P.B.K.)[6] and by the East African Extract Corporation[7]; the P.B.K. method may be taken as typical of these four, which differ in only minor details. They also resemble the A.O.A.C. method in requiring hydrolysis of a purified extract of pyrethrum flowers with alcoholic alkali and removal of extraneous acids from the hydrolysate, after dilution with water and removal of alcohol, by the addition of barium chloride. In the P.B.K. method, a filtered portion of the solution is acidified with hydrochloric acid, and the liberated chrysanthemic acid is extracted with light petroleum. The important difference is that in the A.O.A.C. method sulphuric acid is used for this liberation, precipitating barium sulphate from the excess of barium chloride present. This precipitate must be filtered off before the solvent extraction is carried out. It is well known that the A.O.A.C. method gives results for chrysanthemic acid, and hence "pyrethrin I,"* that are some 10 to 15 per cent. lower than those given by the methods in which hydrochloric acid is used, and this has been ascribed to loss of chrysanthemic acid by occlusion on the barium sulphate precipitate. Mitchell[8] confirmed this, showing that chrysanthemic acid could be recovered from barium sulphate residues (previously leached with water until the washings contained no chrysanthemic acid). It was also pointed out by Beckley and Hopkins[9] that a group of amorphous acids was occluded on the barium sulphate residue, and they suggested that these in turn were responsible for the retention of chrysanthemic acid.

The "mercury reduction" reaction itself leaves much to be desired. It is empirical in nature and gives correct results only by strict adherence to the stated conditions of time, temperature, etc. Further, the factor used to calculate the results is an average value reached as a result of collaborative trial between several laboratories.[10] Evidently, there is not a stoicheiometric relation between the reactants. An attempt to find a more specific means of determination prompted the work described here, which invokes measurement of the infrared absorption of chrysanthemic acid. This has given promise, and the results obtained are in fairly close agreement with those provided by the A.O.A.C. method. It is now clear that the methods in which hydrochloric acid is used, despite previous claims, include and measure some extraneous acids as "chrysanthemic acid." It was nevertheless a surprise to us that agreement between the infrared and A.O.A.C. results was so close, particularly as it is known that some chrysanthemic acid (though perhaps less than was formerly thought) is lost on the barium sulphate precipitate. It seems evident that the extraneous acids (possibly the amorphous acids claimed by Beckley and Hopkins[9]) are mostly removed with the barium sulphate precipitate; it seems likely, however, that a proportion is not lost, but is recorded as "chrysanthemic acid," and that it is similar in amount to the true chrysanthemic acid that is lost. In other words, it may be that the A.O.A.C. method gives results in fair agreement with those by the infrared method by a summation of errors, positive and negative.

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* Comprising pyrethrin I and cinerin I, but calculated as pyrethrin I—hence the parentheses, which have similar significance for "pyrethrin II" and total "pyrethrins."

Experimental

The infrared spectrum (see Fig. 1) of pure D-*trans*-chrysanthemic acid (isolated after hydrolysis of pyrethrum extract; m.p. 18·5° C; mercury reduction assay 99·9 per cent.), dissolved in carbon tetrachloride, shows an isolated peak at about 9·0 μ, which we selected for purposes of measurement.

Our procedure was to scan the range 8·0 to 9·5 μ, draw a base line between the two troughs at 8·6 and 9·2 μ, measure the percentage transmission (T_0) from the radiation zero to this base line, and also the percentage transmission (T) from the radiation zero to the absorption peak. The absorption (A) was given by $\log_{10} T_0/T$. This method of measurement was adopted to discount, so far as possible, extraneous absorption caused by impurities in the acid. It was shown (see Table I) that the absorption of pure chrysanthemic acid obeyed the Beer - Lambert law over a considerable range of concentrations, the resulting graph being a straight line passing through the origin.

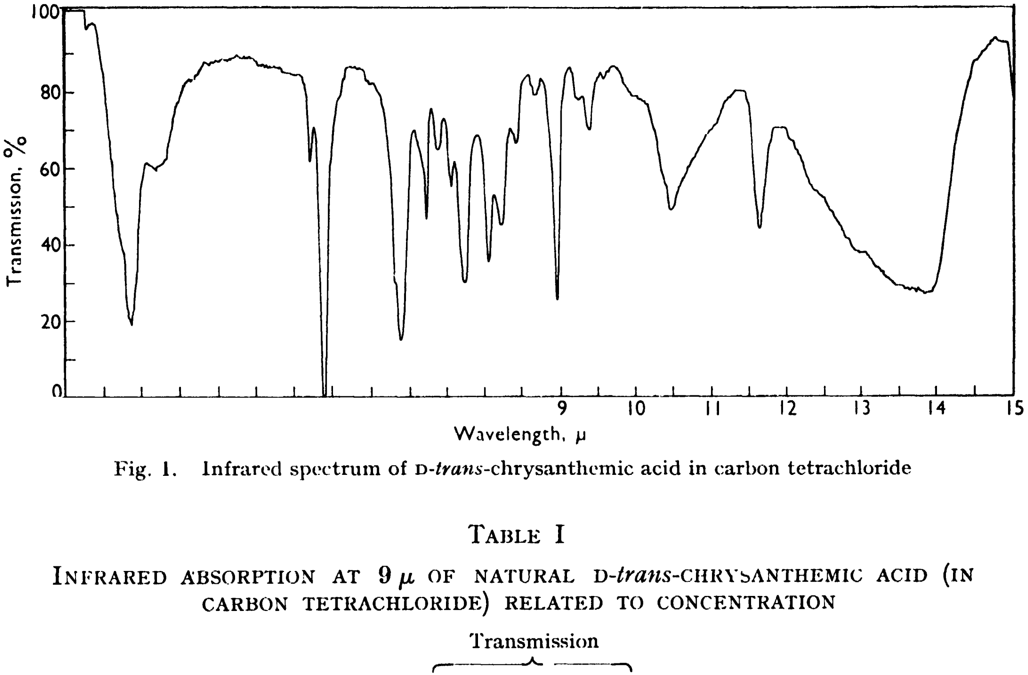

Fig. 1. Infrared spectrum of D-*trans*-chrysanthemic acid in carbon tetrachloride

TABLE I

INFRARED ABSORPTION AT 9 μ OF NATURAL D-*trans*-CHRYSANTHEMIC ACID (IN CARBON TETRACHLORIDE) RELATED TO CONCENTRATION

Concentration, g per 10 ml	Transmission T_0, %	 T, %	Absorption ($\log T_0/T$)
0·0130	95·7	65·5	0·165
0·0234	95·8	52·8	0·259
0·0341	91·0	38·8	0·370
0·0422	91·3	31·7	0·459
0·0572	85·3	20·3	0·623
0·0656	85·9	17·4	0·693
0·0748	82·3	12·5	0·819
0·0886	79·7	9·1	0·943

To apply this method of measurement to the analysis of pyrethrum extract, we used the P.B.K. method[6] to the stage where the chrysanthemic acid had been extracted with light petroleum (boiling range 40° to 60° C). This light petroleum solution was then extracted with four portions (10, 10, 5 and 5 ml) of 0·5 N aqueous sodium hydroxide. The combined alkaline extracts were neutralised to phenolphthalein with concentrated hydrochloric acid, and then a 0·5-ml excess of the acid was added. The acidified solution was then extracted with five portions (3, 2, 2, 1 and 1 ml) of analytical-reagent grade carbon tetrachloride. Each extract was successively strained through anhydrous magnesium sulphate (0·5 g supported on a plug of cotton-wool in a small funnel) into a 10-ml calibrated flask; more solvent was then passed through the magnesium sulphate and into the flask to give a total volume of 10 ml. Infrared measurements were made on this solution as described above with a Perkin-Elmer "Infracord 137" infrared spectrophotometer and 0·1-mm cells. From the measured absorption (A), the weight (x) of chrysanthemic acid contained in the 10 ml of solution was then read off from the graph prepared from the measurements recorded in Table I. "pyrethrin I" was calculated by substitution in the formula—

$$\text{P}_\text{y}\text{I}, \% = \frac{x \times 41 \times 5 \times 100}{21 \times 4 \times w}$$

where x = weight of chrysanthemic acid, in grams, and
w = weight of sample of extract, in grams.

"Pyrethrin II" was determined by the usual procedure, but with the additional modification described by Mitchell and Tresadern[11] to reject extraneous water-insoluble acidic matter.

The A.O.A.C. assays all included the prescribed preliminary chilling in light petroleum solution. With the P.B.K. method, comparisons were made with and without the preliminary treatment in light petroleum solution. Omission of the treatment means that the extract, as received, was subjected directly to hydrolysis.

RESULTS AND DISCUSSION

Table II shows the comparative results obtained for "pyrethrin I" on eleven samples of Kenya pyrethrum extract, ordinary grade. It can be seen that those obtained by the recommended infrared method are significantly lower than those found by the P.B.K. method (mean difference is 11 per cent.), that omission of the preliminary treatment in light petroleum solution causes only slightly higher results by the recommended infrared method, and that the results by the A.O.A.C. method are fairly closely similar to, and generally slightly lower than, those obtained by the recommended infrared method.

TABLE II

COMPARATIVE RESULTS FOR "PYRETHRIN I" IN PYRETHRUM EXTRACTS

	"Pyrethrin I" found by—			
		Recommended method		
Sample	P.B.K. method,[6] %	With preliminary treatment, %	Without preliminary treatment, %	A.O.A.C. method,[3] %
A	13·6	11·9	12·0	11·8
B	13·2	11·6	11·7	11·7
C	13·5	12·1	12·2	11·9
D	13·3	12·0	12·2	11·8
E	13·2	11·3	11·1	11·1
F	13·8	12·0	12·2	12·1
G	13·3	11·5	11·6	11·5
H	14·8	13·5	—*	—*
I	13·4	12·2	—*	—*
J	14·5	12·5	—*	—*
K	13·6	11·7	11·8	11·6
Mean ..	13·4	11·8	11·9	11·7

* Not determined.

Results of comparative "pyrethrin II" assays on several of the same extracts are shown in Table III. As the P.B.K. and A.O.A.C. methods give similar directions for determining "pyrethrin II," it is not surprising that the results obtained are also similar. This also applies when Mitchell and Tresadern's modified method[11] is included, but the results are some 6 per cent. lower because of the rejection of acids insoluble in hot water. Equally, this applies to the recommended method, except when the preliminary light petroleum treatment of the extract is omitted. In that case, the results are rather intermediate, being about 4 per cent. lower than those to which the modification has not been applied. When both the preliminary treatment and the modification to the "pyrethrin II" method are omitted, the mean results for "pyrethrin II" are some 5 per cent. higher than those by the P.B.K. method.

TABLE III

COMPARATIVE RESULTS FOR "PYRETHRIN II" IN PYRETHRUM EXTRACTS

"Pyrethrin II" found by—

Sample	P.B.K. method,[6]		Recommended method with preliminary treatment	Recommended method without preliminary treatment		A.O.A.C. method,[3]	
	(*i*), %	(*ii*), %	(*ii*), %	(*i*), %	(*ii*), %	(*i*), %	(*ii*), %
A	11·6	11·0	11·0	—*	10·9	—*	—*
B	11·9	11·0	11·0	—*	11·3	11·7	11·0
C	11·2	10·5	10·5	11·9	11·0	11·2	10·6
D	11·0	10·3	10·3	—*	10·7	10·8	10·2
E	11·3	10·7	10·7	11·8	11·0	11·2	—*
F	12·3	11·5	11·5	12·8	11·5	12·2	—*
G	11·7	11·3	11·4	12·4	11·6	11·9	—*
Mean ..	11·6	10·9	10·9	12·2	11·1	11·5	10·6

(*i*) = Omitting Mitchell and Tresadern's method.[11]
(*ii*) = Including Mitchell and Tresadern's method.[11]

* Not determined.

From these results it is clear that the preliminary treatment has no particularly significant effect on the results for "pyrethrin I," but its omission does give somewhat higher results for "pyrethrin II," even when steps have been taken to remove interfering water-insoluble acidic matter. To facilitate comparison, Table IV shows the figures for "total pyrethrins" given by the various methods, individually modified as before. On the whole, it would appear to us that inclusion of the preliminary treatment is not warranted. It occupies extra laboratory time; by involving extra manipulation it increases the risk of error; and it does not have a particularly marked effect on the results. Omission of this step would probably introduce some small positive errors; this would not seem to us to matter unduly in a method designed to control commercial transactions in pyrethrum extract. Without the preliminary treatment, but including the purification step in the "pyrethrin II" determination, the results are close to those given by the current A.O.A.C. method.

It having been noted that the ratio of "pyrethrin I" to "pyrethrin II" is close to unity, the idea (advanced some years ago by Mitchell[12]) of standardising only in terms of "pyrethrin I" may be worth further consideration. Its adoption would certainly simplify and shorten the assay.

TABLE IV

COMPARATIVE RESULTS FOR TOTAL "PYRETHRINS" IN PYRETHRUM EXTRACTS

Total "pyrethrins" found by—

Sample	P.B.K. method[6]		Recommended method with preliminary treatment,	Recommended method without preliminary treatment		A.O.A.C. method[3]	
	(*i*), %	(*ii*), %	(*ii*), %	(*i*), %	(*ii*), %	(*i*), %	(*ii*), %
A	25·2	24·6	22·9	—*	22·9	—*	—*
B	25·1	24·2	22·6	—*	23·0	23·4	22·7
C	24·7	24·0	22·6	24·1	23·2	23·1	22·5
D	24·3	23·6	22·3	—*	22·9	22·6	22·0
E	24·5	23·9	22·0	22·9	22·1	22·3	—*
F	26·1	25·3	23·5	25·0	23·7	24·3	—*
G	25·0	24·6	22·9	24·0	23·2	23·4	—*
Mean ..	25·0	24·3	22·7	24·0	23·0	23·2	22·4

(*i*) = Omitting Mitchell and Tresadern's method.[11]
(*ii*) = Including Mitchell and Tresadern's method.[11]

* Not determined.

A parallel investigation of these problems has been made by Brierley and Brown.[13] They also examined the possibilities of an infrared assay involving use of the absorption maximum at 9 μ. Further, they examined a method involving isolation of chrysanthemic acid by steam-distillation along the lines of the Seil method,[14] but under conditions that avoid hydration, and consequent loss, of part of the chrysanthemic acid.[15,16,17] They also applied a silica gel column-chromatographic procedure that afforded them relatively pure chrysanthemic acid in one fraction, and in another a mixture of other acids that reduced the mercury reagent, but without the usual characteristic colour changes.

Each of these last two methods gave them closely similar results on any given sample, results that were also in fairly close agreement with those given by the A.O.A.C. method, but the authors made no firm recommendation for the adoption of any of these methods. However, Brierley and Brown believe, probably correctly, that these results comprise not only the true active principles, but also any "false" pyrethrins and free chrysanthemic acid present in the original extract. To overcome this, they propose to use the preliminary chromatographic purification with alumina suggested some years ago by Brown, Phipers and Singleton.[18] This further lowers the results, so that their average final results were some 10 per cent. lower than those by the A.O.A.C. method or 25 per cent. lower than those by the P.B.K. version.

TABLE V

COMPARATIVE RESULTS ON A SAMPLE (B) OF PYRETHRUM EXTRACT

Preliminary treatment in light petroleum (boiling range 40° to 60° C) was carried out in all tests

	Steam-distillation[13]	Infrared	Chromatography[13]	A.O.A.C.[3]
"Pyrethrin I," % ..	11·5	11·6	11·3†	11·7
"Pyrethrin II,"* % ..	11·1	11·0	11·1	11·0
Total "pyrethrins," % ..	22·6	22·6	22·4	22·7

* Purified by Mitchell and Tresadern's method.[11]

† This represents only "fraction A" as defined in the method.[13] The acidic matter was recovered after titration. Infrared measurement then showed 11·5 per cent., in good agreement with the direct infrared measurement. In another similar experiment, the recovered material, measured by "mercury reduction," also amounted to 11·5 per cent.

Using our Sample B, we confirmed that the steam-distillation method,[13] the silica gel chromatographic method[13] and the infrared method gave results in close agreement, in turn agreeing well with those obtained by the A.O.A.C. method (see Table V). We also obtained a useful cross check by measuring the material comprising "Fraction A" by the infrared and by the "mercury reduction" methods. The results were all in good agreement (see Table V).

However, we question the need for applying to extracts the initial chromatographic purification procedure (Brown, Phipers and Singleton[18]), for the reasons stated below—

1. It would extend an already lengthy analytical procedure (which we would propose to shorten by eliminating even the relatively simple preliminary treatment now applied).

2. Unless extremely carefully controlled, this chromatographic procedure can result in loss of pyrethrins, cinerins or both. This was observed during a collaborative trial of the method some years ago.[19]

3. It seems scarcely justified to strive after greater accuracy by this means, when one reflects that the factors used for calculating the amounts of "pyrethrin I" and "pyrethrin II" are derived from the molecular weights of the pure pyrethrins. In other words, no cognisance is taken of the presence of the two cinerins. Because it is not unlikely that the relative proportions of these four active principles vary in different batches of pyrethrum flowers or pyrethrum extracts, one can expect that the figures reported will not always be directly proportional to the over-all biological activity in any event.

We consider that the method adopted need not strive for greater accuracy than is needed to control commercial transactions in pyrethrum. If we have to abandon the P.B.K. method —a step that would now seem to be justified—we would consider it a good opportunity to discard all variants of the methods incorporating the empirical "mercury reduction" procedure. Instead, we would like to substitute a method providing measurement of chrysanthemic acid by a more reliable procedure. We consider that the infrared method described here meets this requirement sufficiently well for the purpose. Application of the process to eliminate interfering water-insoluble acidic matter suffices, again in our opinion, to correct the "pyrethrin II" value sufficiently for practical purposes (though we have reason to believe that extraneous acids are still being measured, hence falsely raising the calculated results for "pyrethrin II").

In an earlier publication[20] one of us suggested that greater accuracy was being expected of the pyrethrum analyst than was reasonable. Because of the high value of pyrethrum, a relatively small difference in analytical results can represent a large sum of money as between buyer and seller. For example, if the buyer's analyst returns 24 per cent. of total pyrethrins as his result on an extract offered as containing 25 per cent., the difference to the uninitiated appears considerable; it would almost certainly represent a lot of money in a commercial transaction. Yet such a difference is evidently only 4 per cent.—well within the usual limits of analytical error—particularly when a rather complex procedure is involved. It is only when inter-laboratory differences consistently occur in one direction that the position can really be regarded as unsatisfactory. Experienced pyrethrum analysts, with the methods currently in use, usually secure results agreeing within the limits of ±5 per cent. In putting forward this proposed new method, we do not suggest for one moment that it is likely to lead to better inter-laboratory agreement than this. We only advance it because we think it gives results nearer the truth, and because it uses a more realistic measurement than the "mercury reduction" procedure.

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Determination of Rubber in the Vegetative Parts of *Hevea brasiliensis*

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A rapid method is described for determining rubber in the various vegetative parts of *Hevea brasiliensis*, based on an iodimetric procedure. A statistical study of the results of plant analyses has shown the method to be unbiased; its precision is shown to vary according to the vegetative part analysed and to lie between 1 per cent. for leaves and petioles and 6 per cent. for brown stems. In the preparation of plant tissues for analysis by the proposed method, storage of samples was found to be a critical factor.

An accurate and sensitive method for determining the rubber content of the various vegetative parts of the rubber tree is needed, both to measure the effect of experimental treatments on latex formation,[1] and as a possible means of assessing the potentialities of newly-bred varieties at an early stage in growth. Defects in existing gravimetric methods[2,3,4] are that they are either too time-consuming for routine analyses, or that they guard insufficiently against the possibility of interference by other substances[5]; all require large amounts of material for analysis, which is a serious objection when tissues containing small amounts of rubber have to be analysed.

The efficiency with which rubber is extracted from vegetative tissues, before its determination, is another problem that has received insufficient attention, particularly in the way in which interference by non-rubber substances reduces the efficiency of extraction. Spence and Caldwell[3] have shown, however, that successive extractions with hot water and acetone are essential, both to remove interfering substances and to prepare plant tissue for the subsequent extraction of rubber by benzene; this "triple-solvent extraction" has been used as a starting point for a study of this particular problem.

The method described here has been made considerably more rapid than those referred to above by employing more efficient means of eliminating interfering substances, and its sensitivity has been increased by using an iodimetric procedure[6] for determining rubber. To gauge the effectiveness with which rubber is extracted and interferences are removed, a statistical study similar to that described by Youden[7] has been carried out on the results of analyses of *Hevea brasiliensis* by the proposed method.

METHOD

APPARATUS—

The special extractor, condenser, and extraction flask, which are suitable for setting up as multiple units, are shown in Fig. 1; the extractor and condenser were specially made for us by Gallenkamp & Co. Ltd.

REAGENTS—

The reagents must be prepared from pure chemicals and distilled water.

Ethanol, redistilled.

Oxalic acid solution, 1 *per cent. w/v, aqueous.*

Purified benzene, containing 1 *per cent. w/v trichloroacetic acid*—Purify the benzene by shaking it with concentrated sulphuric acid and by washing it four times with water; then filter the benzene, and distil at 80° C, the first and the last 50 ml out of a distillate of 2 litres being discarded. The need for this purification has been discussed in an earlier publication.[6]

***Sodium molybdate solution,* 0·1 *per cent. w/v in* 10 *per cent. v/v hydrochloric acid and* 10 *per cent. v/v acetic acid.**

Potassium bromate solution,* 0·55 *per cent. w/v in* 10 *per cent. w/v potassium bromide.

Sodium borate solution,* 2·5 *per cent. w/v, aqueous.

***Sodium thiosulphate solution,* 0·04 N.**

Iodine solution,* 0·01 N *in* 4 *per cent. w/v potassium iodide.

PROCEDURE—

The procedure described below is suitable for leaves, petioles and stems of *Hevea brasiliensis*.

Put a weighed sample of dry, milled plant tissue containing 5 to 20 mg of rubber between two layers of cotton-wool in an extractor, as shown in Fig. 1. Hang the extractor on the hook of a condenser, and pour 100 ml of ethanol through the extractor into an extraction flask. Lower the condenser into the neck of the flask, place the flask on a flame-proof electric heater, and adjust the rate of boiling to ensure a steady drip of ethanol from the condenser. After 3 hours' extraction, remove the extractor, and leach its contents five times with small amounts of hot ethanol; allow the excess to drain away, but do not allow the sample to dry completely. Then hang the extractor on the hook of another condenser. Lower the extractor into an extraction flask containing 100 ml of distilled water, and heat, the rate of boiling being adjusted to ensure a steady drip of water through the extractor. After 2 hours, remove the extractor, leach with a little ethanol to facilitate removal of water, and dry at 75° C in an oven.

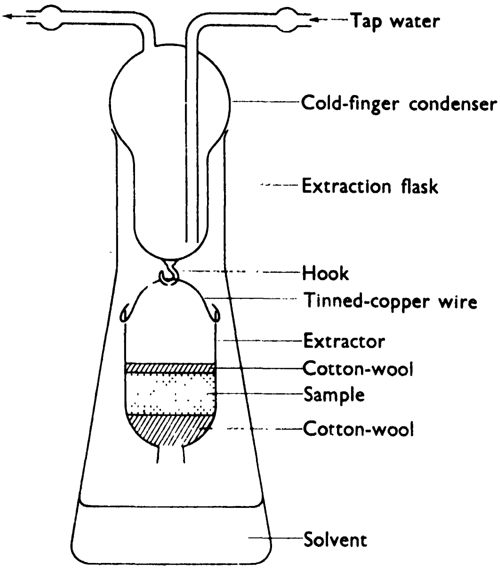

Fig. 1. Apparatus for extracting rubber-bearing tissue with alcohol and water

Transfer the contents of the extractor completely to a 100-ml polythene bottle provided with a screw cap, add 50 ml of benzene, and place in a dark cupboard overnight to ensure swelling of the rubber in the solvent. Shake the bottle vigorously for 45 minutes, and filter some of the contents through a Whatman No. 1 filter-paper into a 25-ml measuring cylinder; transfer the 25-ml portion, without delay, to an opaque stoppered bottle, and add 5 ml of sodium molybdate solution and then exactly 5 ml of potassium bromate solution. Replace the stopper tightly, mix the aqueous and benzene phases thoroughly, and place the bottle in a dark cupboard. After 100 minutes have elapsed, record the temperature of the cupboard, remove the bottle, and quickly add 2 g of potassium iodide to its contents. Replace the stopper tightly, shake the solution thoroughly, and after 5 minutes quickly add 100 ml of sodium borate solution. (A spouted 100-ml calibrated flask permits a rapid addition of sodium borate to be made; it is essential that this flask and all glass apparatus used in the bromination process should be free from grease.) Again shake thoroughly, and add 25 ml of sodium thiosulphate solution with continuous swirling. After 5 minutes, filter about 20 ml of the aqueous phase through a Whatman No. 1 filter-paper (previously moistened with distilled water to ensure that only the aqueous phase passes through), and discard. Filter the remainder of the aqueous phase into a clean dry flask, and titrate a 50-ml portion with standard iodine solution, with starch as indicator. Carry out by the proposed procedure a blank determination on 25 ml of benzene only. Determine the volume of standard iodine solution corresponding to the bromine absorbed by the rubber by multiplying the differences between the two titrations by the total volume of the aqueous phase (135 ml) and dividing by the volume of the portion taken (50 ml).

For the roots of *Hevea brasiliensis*, a preliminary extraction with dilute oxalic acid, as recommended by Willits *et al*,[4] is necessary to remove pectin and gums. Gently boil the weighed sample for 2 hours with 100 ml of oxalic acid in a tall 400-ml beaker, and then transfer the material on to cotton-wool in an extractor by means of a jet of hot water. Leach the contents of the extractor three times with small amounts of hot ethanol, and complete the 3-hour extraction with ethanol as described above. Extract the dried contents of the extractor with benzene; no subsequent extraction with water is required.

CALCULATION OF RESULTS—

For the iodimetric method of determining rubber described above, an empirical factor is needed in order to obtain the weight of rubber from the volume of standard iodine solution used in the titration. By standardising the procedure against purified rubber, Middleton[6] has shown that such a factor (expressed as milligrams of rubber per millilitre of 0·01 N iodine) varies with the temperature of bromination, as shown by the equation given below—

$$\text{Factor at } t^\circ \text{ C} = 0{\cdot}3339 - 0{\cdot}000589\ (t - 25).$$

In order to calculate the rubber content of a sample from the result of an iodimetric titration, it must be taken into account that one half of the benzene extract is taken for bromination, that 50 ml out of 135 ml is taken for the iodimetric titration, and that the factor is expressed as milligrams of rubber per millilitre of standard iodine solution. Consideration of these points gives the equation—

$$\text{Percentage of rubber} = \frac{\text{Volume of 0·01 N iodine} \times \text{factor at } t^\circ \text{ C} \times 0{\cdot}54}{\text{Weight of sample taken}}$$

EFFICIENCY OF THE METHOD

The efficiency with which rubber in plant tissue is determined depends on the effectiveness of the extraction process, on the extent to which interfering substances are removed, and on the way in which the sample is prepared for analysis.

The usual method for removing rubber from plant tissue is by means of an extraction of 16 to 24 hours with benzene in a Soxhlet or similar apparatus.[2,3,4] Preliminary work had

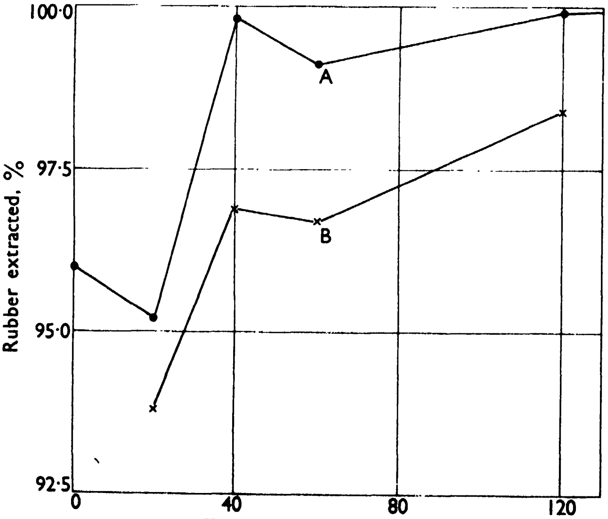

Fig. 2. Efficiency of the shaking method for extracting rubber from plant tissue: curve A, after contact with the solvent overnight; curve B, without contact with the solvent overnight

indicated that shaking with benzene containing a little trichloroacetic acid was a speedier and more efficient method. The effect of allowing rubber-impregnated tissue to remain in contact with the solvent overnight and the effect of varying the time of shaking on the extraction of rubber are shown in Fig. 2. The efficiency of such a method of extraction has also been tested by recovering rubber, after known amounts of latex had been added to plant tissue initially free from rubber; the results are shown in Fig. 3.

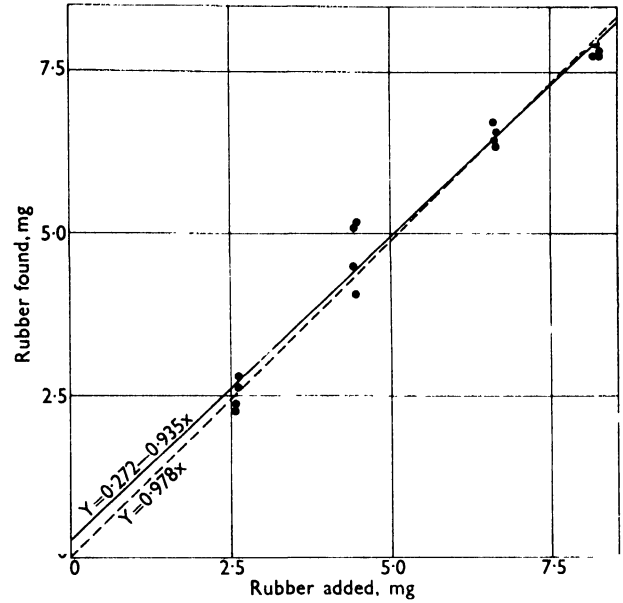

Fig. 3. Recovery of rubber from impregnated Eucalyptus stems

The presence of interfering substances capable of absorbing bromine should cause a positive bias in results obtained for the determination of rubber by the proposed method; conversely, any reduction in the efficiency of the extraction of rubber should produce a negative bias. To investigate these possible sources of error, increasing weights of tissue were analysed, as recommended by Youden[7]; the results are shown in Table I.

Table II shows the effects of different drying temperatures, of milling the material to pass either a 0·5-mm screen (finely ground) or a 1·55-mm screen (coarsely ground), and of subsequently storing the samples.

TABLE I

ANALYSES OF VEGETATIVE PARTS OF *Hevea brasiliensis*

Rubber found as a percentage of oven-dry material

Weight of material, g	Leaves		Petioles		Green stems		Brown stems		Roots	
	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse
0·5	—	—	1·938	1·919	—	—	—	—	—	—
	—	—	1·938	1·937	—	—	—	—	—	—
1·0	0·447	0·443	1·937	1·936	0·591	0·580	0·242	0·232	0·373	0·386
	0·447	0·452	1·928	1·927	0·600	0·571	0·251	0·271	0·373	0·376
1·5	—	—	1·937	1·936	—	—	—	—	—	—
	—	—	1·863	1·924	—	—	—	—	—	—
2·0	0·442	0·456	1·937	1·941	0·582	0·571	0·237	0·242	0·373	0·376
	0·446	0·441	1·947	1·945	0·614	0·575	0·233	0·247	0·382	0·386
3·0	0·437	0·452	—	—	0·585	0·568	0·254	0·242	0·373	0·376
	0·443	0·449	—	—	0·594	0·564	0·279	0·242	0·373	0·376
4·0	0·446	0·452	—	—	0·603	0·535	0·242	0·233	0·371	0·374
	0·451	0·454	—	—	0·603	0·573	0·247	0·235	0·376	0·379
Mean	0·445	0·451	1·928	1·933	0·597	0·567	0·248	0·243	0·374	0·379

TABLE II

EFFECT OF VARIOUS TREATMENTS ON THE DETERMINATION OF RUBBER IN *Hevea brasiliensis*

Results expressed as percentages of oven-dry material

Vegetative part	Finely ground			Coarsely ground	
	A	B	C	A	B
Leaves	0·440	0·387	0·389	0·451	0·386
Petioles	1·933	1·881	1·848	1·932	1·879
Green stems	0·590	0·479	0·471	0·566	0·476
Brown stems	0·245	0·218	0·213	0·234	0·216
Roots	0·374	0·373	0·375	0·377	0·376

A = Material dried at 55° C and analysed immediately.
B = Material dried at 55° C and analysed after 14 weeks.
C = Material dried at 55° C, stored for 14 weeks and analysed after drying at 85° C.

DISCUSSION OF THE METHOD

Of the various methods available for analysing rubber-bearing plants, the triple-solvent extraction of Spence and Caldwell[3] has been referred to[5] as the first truly quantitative procedure; it specifies successive extractions with hot water and acetone, with subsequent extraction of rubber with warm benzene in a Soxhlet-type apparatus. Even in a simplified form[4] the procedure is lengthy, at least 3 days being needed to complete a determination. Recently, Meeks *et al.*[5] have criticised this type of extraction with benzene as inefficient and have described a method in which rubber is extracted by shaking with benzene containing trichloroacetic acid, without any pre-treatment with water and acetone.

Preliminary work here on *Hevea brasiliensis* had nevertheless confirmed that pre-treatment with hot water and acetone or ethanol was essential, both to remove interfering substances and also to disintegrate plant tissues sufficiently to permit the extraction of rubber by benzene to proceed effectively. On the other hand, these studies had also confirmed that shaking with benzene containing trichloroacetic acid was a more effective method for bringing rubber into solution than extraction in a Soxhlet-type apparatus. Trichloroacetic acid helps in dispersing gel rubber and in reducing the viscosity of rubber solutions in benzene; neither of these advantages is possible with a Soxhlet-type extraction.

To assess the efficiency of the shaking method for extracting rubber from plant tissues, milled stems of the tree *Eucalyptus deglupta*, which contains no rubber, were impregnated with a definite amount of *Hevea* latex of known composition, to give a mixture the rubber content of which could be calculated. The effect of different times of shaking and of allowing the material to remain in contact with benzene overnight before shaking is shown in Fig. 2. A statistical examination of duplicate determinations, from which these plotted means were obtained, has shown that, compared with direct shaking, a significantly greater amount of rubber was extracted after the overnight treatment. With the latter procedure, a shaking time of about 45 minutes has been specified in order to ensure a recovery not significantly different from 100 per cent. This might be expected, since it has already been shown[8] that the initial stage of the dispersion of *Hevea* rubber in benzene is a slow disintegration into small swollen particles that require considerable further shaking to bring them into solution.

ACCURACY OF THE METHOD

In assessing the accuracy of the proposed method, no attempt has been made to compare the results obtained by it with any obtained by the other methods mentioned. The reasons for not doing so are that in the methods that make adequate provision for removing interfering substances[2,3,4] the procedure for extracting rubber, namely in a Soxhlet apparatus, is considered to be inefficient and, conversely, that in the method[5] by which rubber could be effectively extracted insufficient attention has been paid to removing interference, at least as far as the analysis of the rubber tree is concerned.

Youden[7] has, however, discussed an alternative way of testing an analytical procedure for bias. Accordingly, different weights of various vegetative parts of *Hevea brasiliensis* have been analysed by the proposed procedure: the results of duplicate determinations on both fine and coarse material are shown in Table I. A statistical examination of the relation between rubber hydrocarbon and weight of material taken for analysis has shown that least-squares regression lines do not differ significantly from straight lines passing through the origin. Such a result clearly demonstrates that there can be no constant bias, otherwise the lines would make significant intercepts on the ordinate axis.

This demonstration does not prove that there is no variable bias proportional in amount to the rubber content of the material being analysed. To test this possibility, milled stems of the Eucalyptus tree, impregnated with a known amount of rubber, were analysed by the proposed method, and rubber found (y) was plotted against rubber added (x), as shown in Fig. 3; a regression analysis of the results is summarised in Table III. Each regression is highly significant and accounts for 97 per cent. or more of the total variation; the best fit is given by the least squares equation (a), but the residual variation about this regression line is not significantly smaller than that found for equation (b), which represents a line passing through the origin with a slope of 0·978. This confirms that the procedure has no significant constant bias and gives 97·8 per cent. as an estimate for the recovery of rubber. Table III also shows that the residual sum of squares for equation (b) is not significantly smaller than that for equation (c), so that the estimated recovery of 97·8 per cent. does not differ significantly from 100 per cent., and therefore no significant variable bias is established.

The variability of the results obtained with impregnated Eucalyptus is considerably greater than that found for the analyses of *Hevea*; but this is considered to be a reflection of the difficulties encountered in adding latex quantitatively to Eucalyptus in such a way that the precipitated rubber could be uniformly dispersed through the milled material.

PRECISION OF THE METHOD

A statistical examination of the results in Table I is shown in Table IV and indicates that there are no significant differences in the percentages of rubber found when different

TABLE III

REGRESSION ANALYSIS FOR THE RECOVERY OF RUBBER FROM IMPREGNATED EUCALYPTUS STEMS

Regression equation	Residual sum of squares	Degrees of freedom	Differences between the residuals: 1 d.f.	Differences between the residuals: 2 d.f.	Test of significance
(a) $y = 0{\cdot}272 + 0{\cdot}935x$	1·5937	14			
$(\pm 0{\cdot}232)$ $(\pm 0{\cdot}040)$			0·1551		$F_{1,14} = 1{\cdot}36$ n.s.
(b) $y = 0{\cdot}978x$	1·7488	15		0·4208	$F_{2,14} = 1{\cdot}85$ n.s.
$(\pm 0{\cdot}015)$			0·2657		$F_{1,15} = 2{\cdot}28$ n.s.
(c) $y = x$	2·0145	16			

n.s. = Not significant.

TABLE IV

COMPONENTS OF VARIANCE AND COEFFICIENTS OF VARIATION FOR EACH VEGETATIVE PART OF *Hevea brasiliensis*

Source of variation	(d.f.)	Leaves	Petioles	Green stems	Brown stems	Roots
			(Mean squares of × 10^6)			
Fine *vs*. Coarse	(1)	132*	105 n.s.	3481**	110 n.s.	77 ($P < 0{\cdot}1$)
Weights	(3)	21 n.s.	531 n.s.	75 n.s.	221 n.s.	19 n.s.
Interaction	(3)	18 n.s.	337 n.s.	173 n.s.	247 n.s.	6 n.s.
Duplicates..	(8)	16	372	173	140	21
			(Components of variance × 10^6)			
Fine *vs*. Coarse	σ_3^2	14	0	413	0	7
Weights	σ_2^2	1	40	0	0	0
Interaction	σ_1^2	1	0	0	54	0
Duplicates..	σ_0^2	16	372	173	140	21
			(Coefficient of variation for a single determination)			
Mean per cent. rubber = $\bar{x}$ ..		0·4478	1·9304	0·5819	0·2456	0·3764
$S^2 = \sigma_0^2 + \sigma_1^2 + \sigma_2^2$ = Variance of a single determination ..		0·000,018	0·000,412	0·000,173	0·000,194	0·000,021
Coefficient of variation = $\frac{100S}{\bar{x}}$		0·95%	1·05%	2·26%	5·67%	1·22%

n.s. = Not significant. * Significant at the 5% level. ** Significant at the 1% level.

weights of the various vegetative parts are taken for analysis. This confirms the conclusions reached from the regression study of the results in Table I (see p. 547).

The examination also gives the coefficient of variation for a single determination on any sample of tissue for the five vegetative parts, and they range from about 1 per cent. for leaves and petioles to nearly 6 per cent. for brown stems. Table IV shows that there are differences in the results for rubber determined in finely and coarsely ground samples, both for leaves and for green stems, but, although the differences are significant, an examination of Table I will show that they are not of great practical importance.

PREPARATION OF SAMPLES OF PLANT MATERIAL FOR ANALYSIS

When tissues of rubber-bearing plants are milled, the formation of rubber agglomerates causes uneven distribution of rubber in the milled sample, renders subsequent extraction of rubber more difficult, and, when large amounts of rubber are present, may block up screens in the mill itself. As pointed out by other investigators[3,4] these disadvantages are greater with grinding mills than with hammer mills, and it has been found here that for samples of *Hevea brasiliensis* a beater-cross mill manufactured by Christie and Norris gives good results.

Meeks *et al.*[5] claim that, in preparing samples for analysis, crushing between rolls is more effective than milling, on the assumption that rupturing cell walls mechanically is an important preliminary to the extraction of rubber. For *Hevea brasiliensis* such pre-treatment has been found to hinder subsequent extraction of rubber, possibly because pressing gums, resins and rubber into intimate contact causes mutual protection against removal by solvents; thus rubber would be expected to protect gums and resins against removal by water and alcohol, and in turn gums and resins would hamper the extraction of rubber by benzene.

Table II shows the effect of various operations used in preparing samples for analysis. It will be seen that a marked reduction in the amount of rubber extracted from all vegetative parts, except roots, is produced by storing samples for 14 weeks. The results also confirm that the difference between finely ground and coarsely ground samples is unimportant and that the material can be dried at 55° or 85° C without appreciably changing the apparent rubber content.

CONCLUSIONS

A rapid and sensitive method is described for determining rubber in the various vegetative parts of *Hevea brasiliensis*; it is based on a new iodimetric procedure for rubber hydrocarbon. Although it has been developed specifically for the analysis of the tissues of this tree, it seems probable that the method could also be applied to the analysis of the tissues of other rubber-bearing plants.

A statistical examination of the results of analyses of various vegetative parts of the rubber tree indicates that within the limits tested the degree of fineness to which samples are ground, the temperature at which samples are dried and the weight of sample taken are not critical factors in the preparation of samples for analysis. The results indicate, however, that an important reduction in the amount of rubber extracted can be caused by storing samples for 14 weeks. The results also show an absence of bias in all the determinations, which, together with a prior demonstration that rubber is efficiently extracted, shows that the determination of rubber by the proposed method is accurate. The precision with which determinations can be carried out is given, and is shown to vary considerably with the vegetative part taken for analysis.

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A Modular Gas-chromatography System for analysing Exit Streams from Reactors

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The system described was designed primarily for analysing instantaneous samples obtained from experimental reactors. The samples might have a dew-point up to 150° C and might contain up to 90 per cent. of water.

The apparatus takes a sample from a vapour stream above its dew-point and analyses it gas chromatographically. The design allows many different arrangements, which permits the analysis to be varied to meet the requirements of a particular problem. By stream switching and the use of multiple columns complete analyses from permanent gases to materials having high boiling-points can be carried out, or one or more components of interest can be isolated and determined. Unwanted components are either back-flushed or eluted in or near the air peak.

The two basic modules are a thermostatically controlled oven and an electrical and pneumatic control box. These two can be stacked in any order and number. There are six basic gas-chromatographic components that can be arranged in the oven module or modules in any combinations fitting into the available space.

The basic modules and components and several arrangements for carrying out simple and more or less complicated analyses are described.

The development of new processes in the chemical industry requires swift and accurate means of analysing complex mixtures from experimental reactors. The samples have to be taken from vapour streams containing many components, and analysis may be difficult and time-consuming. The dew-point of the vapour stream may also be high, which complicates the problem of handling the sample. In such circumstances a rapid method of analysis would evidently help to speed up research on a particular subject.

The analytical technique that seemed to offer the best chance of solving this problem was gas chromatography, provided that the requirements listed below could be met.

1. In order to simplify the analytical problem the sample must be taken in a single phase above its dew-point. Sample temperature may, therefore, be as high as 200° C.
2. The sample volume must be accurately reproducible.
3. The sample must be truly representative of the material being analysed.
4. The system must be capable of dealing with samples containing large proportions of steam.
5. The system must be capable of analysing mixtures containing such gases as nitrogen, oxygen, carbon monoxide and methane, together with vapours having dew-points up to 150° C.
6. Because of the rapidly changing nature of research projects, the equipment must be extremely flexible and capable of being readily adapted so that different problems can be studied.
7. The equipment must not be too large and must be simple to operate.

POSSIBLE APPROACHES TO THE PROBLEM

The usual methods of analysing mixtures of materials having a wide range of boiling-points are either to use several columns and detectors in series or to heat the column during the analysis according to a pre-determined temperature programme. Commercial apparatus of both types was considered to see if it would meet our requirements, but none was satisfactory because—

(*a*) the sampling systems could not be operated at 200° C;

(*b*) the systems could not deal with samples containing large amounts of water;

(*c*) the systems were inflexible and could not easily be modified to solve new problems.

It became apparent that if one had a sample valve capable of operating at 200° C, and thermostatically controlled oven modules that could be interconnected, then one could arrange valves, detectors and columns in the modules as required by the gas chromatography problem. Further, if samples could be taken simultaneously and analysed by different columns operating in parallel then the problem of complete analysis of the sample could be solved.

PRACTICAL SOLUTION TO THE PROBLEM

Examination of the Consolidated Electrodynamics Corporation laboratory chromatograph indicated that the basic elements of this equipment could be used in a modular design, and the re-design of the apparatus to our specification was undertaken by Elliott Bros. (London) Ltd., the British Licensees.

The modular system provides the facilities listed below—

(*i*) The sample can be taken in the vapour phase at any temperature from 50° to 200° C, and the sample valve can be maintained, if required, at a temperature different from that of the columns.

(*ii*) Water or unwanted high-boiling fractions can be rejected by the use of a pre-column that can be back-flushed.

(*iii*) Parallel sampling and analysis can be achieved by mechanical linkage of the sample valves.

(*iv*) The use of a constant-mass flow system permits flow switching operations to be carried out without permanent base-line and calibration changes.

(*v*) The system is completely flexible, as the components of the modules can be dismantled and re-arranged in any manner required.

DESCRIPTION OF THE MODULAR GAS-CHROMATOGRAPHY SYSTEM

The system consists of two basic modules, a gas-control and bridge power-supply module and a thermostatically controlled oven module. The six basic components that can be arranged in the oven module are—

1. 6-Port vapour sampling valve.
2. 10-Port vapour sampling valve.
3. Pre-cut and back-flushing column.
4. Analytical column.
5. Detector.
6. Liquid-injection sampling device.

GAS-CONTROL AND BRIDGE POWER-SUPPLY MODULE—

The gas-control system is based on a constant-mass flow controller with a constant upstream pressure (manufactured by A.E.I., Harlow, type 63BUL) so that constant flow is maintained, even if changes in resistance occur downstream of the controller. Hence, columns can be switched in and out with no more than a short-term disturbance of the base line or change in calibration of the detector.

The bridge power supply delivers 6 to 12 volts d.c. to the detector unit. A range switch with 7 steps from 1 × to 1000 ×, a polarity reversal switch and a bridge-balance fine control, are incoporated. Terminals are provided so that an external battery source can be used if desired.

THERMOSTATICALLY CONTROLLED OVEN MODULE—

This is shown in Fig. 6. It consists of a heavy cast-aluminium frame with attached slab heaters, located in a thermally insulated case. The temperature is controlled in the range 50° to 250° C by a thermostat set by a lockable dial on the front. The frame is bilaterally symmetrical so that sample valves or detectors can be fitted at either end of the frame, and the frame is drilled and tapped with all the necessary fixing holes for this purpose. At the rear of the case provision is made for 6 gas entries on either side.

The sample valve can be operated from the front of the case, and, if necessary, valves fitted at each end of the aluminium frame can be mechanically linked to obtain parallel sampling facilities. Provision is made for fitting a liquid-injection device in the centre of

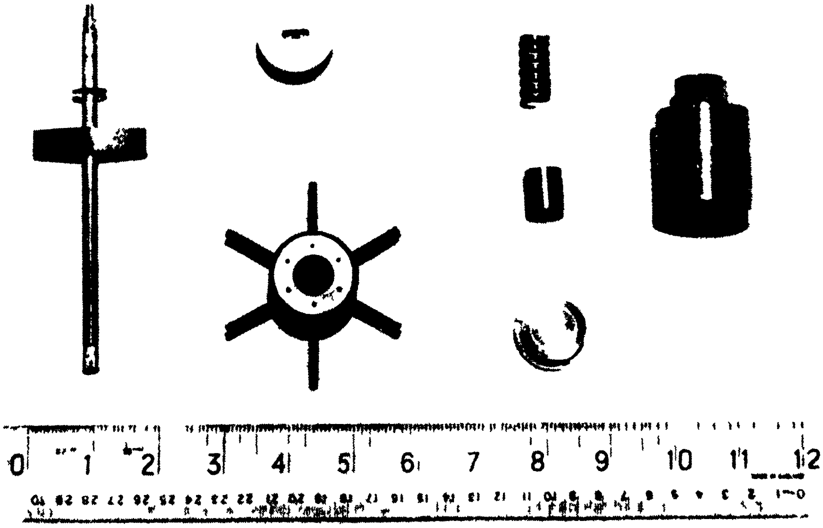

Fig 1 Parts of the 6 port sample valve

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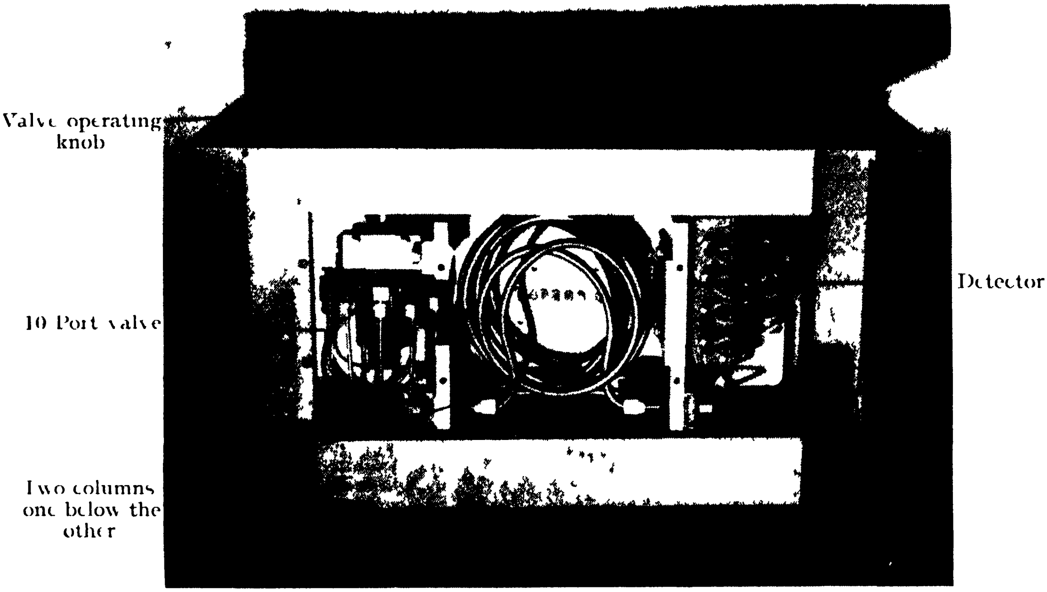


Fig 6 Oven module with cover removed seen from the top—containing 10 port valve two columns and detector

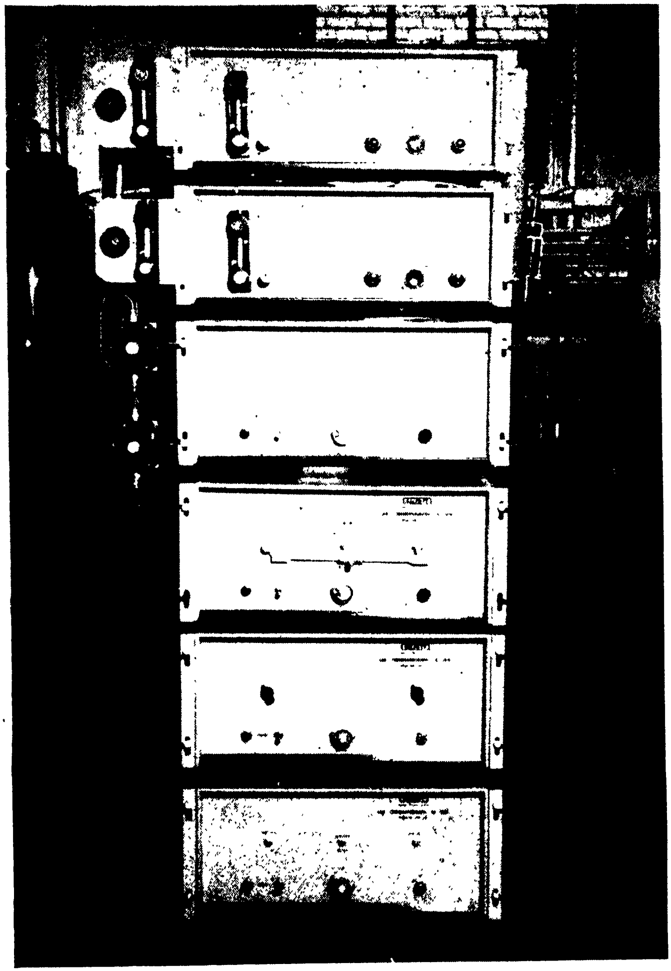


Fig. 12. Arrangement of apparatus set up as shown in Fig. 11, viewed from the front

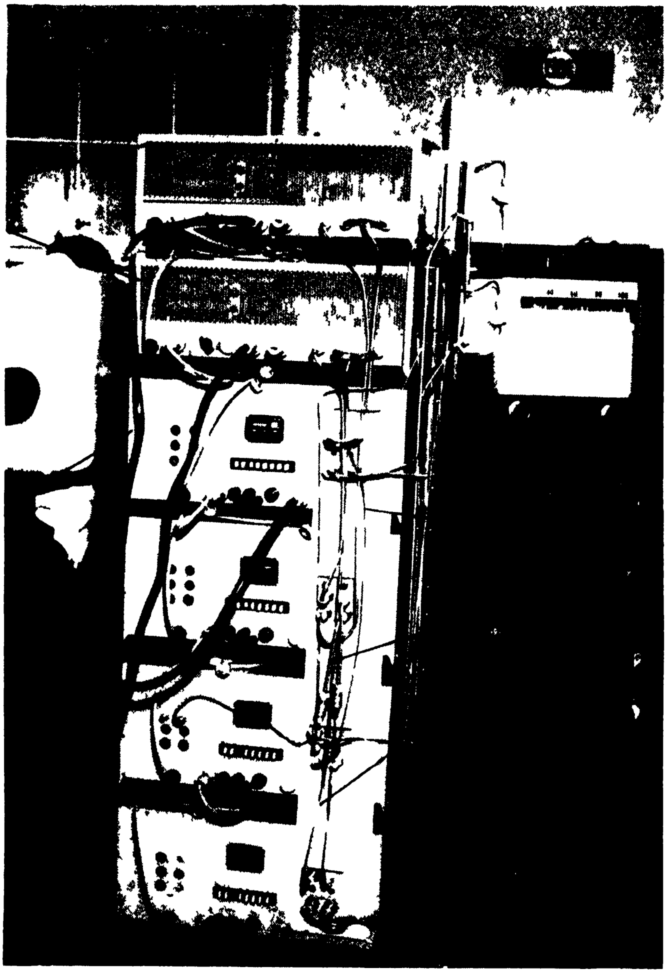


Fig 13 Arrangement shown in Fig 12 viewed from the rear—heating cover for external connections removed

the front panel. All gas connections are made at the rear of the box, and all major controls, except heater switches, are at the front. The gas connections (except for the columns) are ⅛-inch Ermeto compression olive-type safety couplings throughout the system (manufactured by British Ermeto Corporation Ltd., Maidenhead, Berks.), as experience has shown that this size and type is satisfactory.

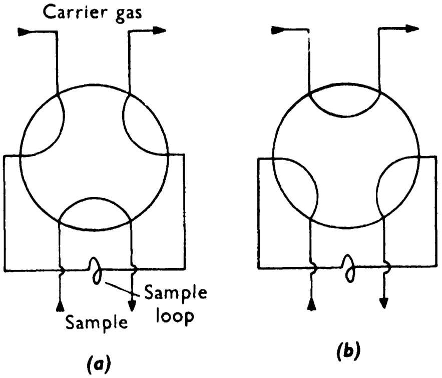
The boxes are designed to stack either locked together or mounted on a frame that allows individual removal of boxes. They can be stacked in any order.

GAS SAMPLING VALVE—

This valve, which can be operated at temperatures up to 200° C and has a reproducibility of ½ per cent., is really the heart of the system. It is made by Elliott Bros. to the C.E.C. design.[1] The components of the valve are shown in Fig. 1, the 6-port and 10-port valves differing only in the number of ports. The valve consists of two faces drilled with the necessary holes and polished flat to within 0·5 wavelength of the cadmium line at 6438·47 Å. One face is made of tungsten carbide and the other, softer, face is made of Niresist—a corrosion-resistant cast nickel - iron alloy. The faces of the valve are held together by a spring, the elastic constants of which are unaffected by repeated use over the entire temperature range. Rotation of the valve switches the gas streams. The operation of the 6-port valve when sampling is shown in Fig. 2. Fig. 3 shows the 6-port valve circuit diagram when the valve is used for pre-cutting and back-flushing. Fig. 4 shows the circuit diagram of the 10-port valve for sampling, with the subsequent operation of pre-cutting and back-flushing.

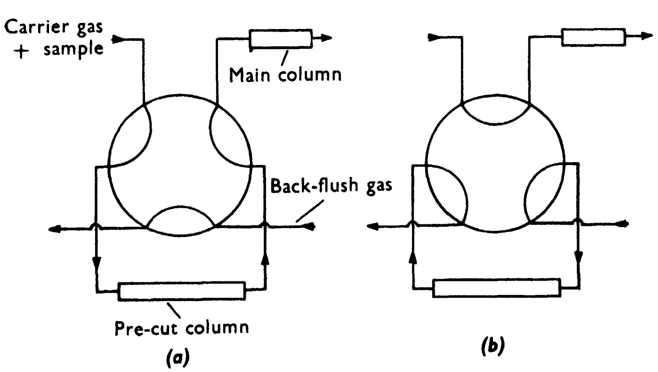
It will be noted that, during all analyses, the flows of sample and carrier gas are continuous, except for the momentary interruption occurring during rotation of the valve.

Lubrication of the valve—A trace of lubricant on the faces is necessary for satisfactory operation. This lubricant must be chemically inert and must have extremely low vapour



(a) (b)

Fig. 2. 6-Port valve used for sampling: (*a*) "s" position—deliver; (*b*) "p" position—refill



(a) (b)

Fig. 3. 6-Port valve used for back-flushing: (*a*) "s" position; (*b*) "p" position—pre-cut

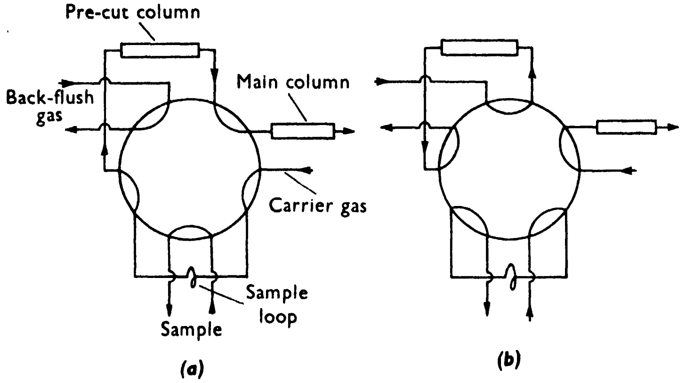


Fig. 4. 10-Port valve used for sampling and back-flushing: (**a**) "s" position—sample on; (**b**) "p" position—pre-cut + sample loop refilled

pressure at the highest temperatures of operation. It must also retain its lubricating properties after prolonged use at high temperatures.

The lubricant finally found to be most suitable for the faces of the valves was Molyspray-Kote *plus* Midland Silicone Valve Seal A. Only the merest trace of the lubricant is required on the faces. After it had been lubricated with this mixture, the valve was operated 600 times at a temperature of 200° C and was still leak-free at the end of this test. The working pressure during the test was 30 lb per sq. inch.

COLUMNS—

The columns are made from ¼-inch outside diameter stainless-steel tube, and are coiled on a winding jig after they have been filled. They can be fitted in an oven module in lengths ranging from 6 inches to a combined length of 40 feet. The columns are attached by ¼-inch Ermeto couplings.

DETECTOR—

This is a thermal conductivity type with four bridge arms of coiled nickel alloy wires inserted in a solid metal block. The filaments are mounted on screwed plugs and are readily

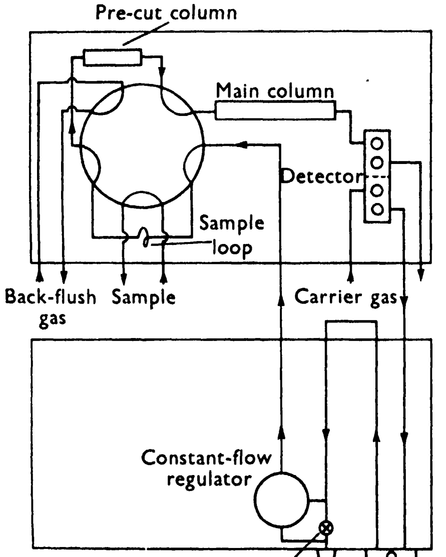


Fig. 5. Simplest arrangement for sampling, back-flushing and analysis

replaced. Gas connections to the detectors are now made by stud couplings. The detectors can be mounted on either side of the thermostat frame.

LIQUID-INJECTION DEVICE—

This is an attachment that can be fitted so that the system can be used if it is required to analyse liquid samples that can be injected with a syringe. For this purpose a gas-control module with a constant-pressure control system should be used, unless the injection system is completely leak-free.

SOME TYPICAL ARRANGEMENTS OF THE MODULES

An example of one of the simpler arrangements is shown in Fig. 5. It comprises one control module and one thermostatically controlled oven box containing a 10-port valve, detector, pre-cut column and analytical column. Fig. 6 shows the oven box with cover removed.

A slightly more complex arrangement comprises one gas-control module and two oven modules. Two tandem columns are included, in which the column order can be reversed without changing the flow direction through the column. The valve arrangement is shown in Fig. 7. The effect of this arrangement is shown in Fig. 8, and it can be seen that the result is to speed up the analysis of the mixture when there are large differences in retention volume between early and late peaks. The less volatile components are eluted first.

Fig. 9 shows a similar arrangement in which prior removal of water and materials having extremely high boiling-points has been effected.

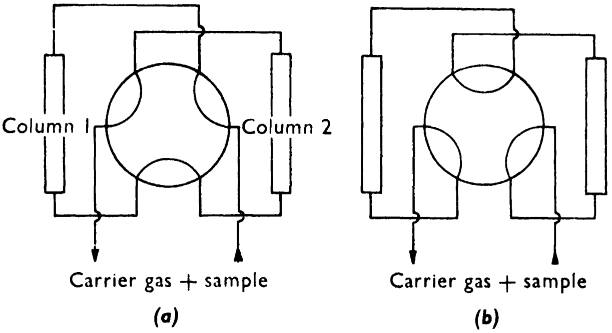


Fig. 7. Valve arrangement for reversing column order: (*a*) column 1 → column 2; (*b*) column 2 → column 1

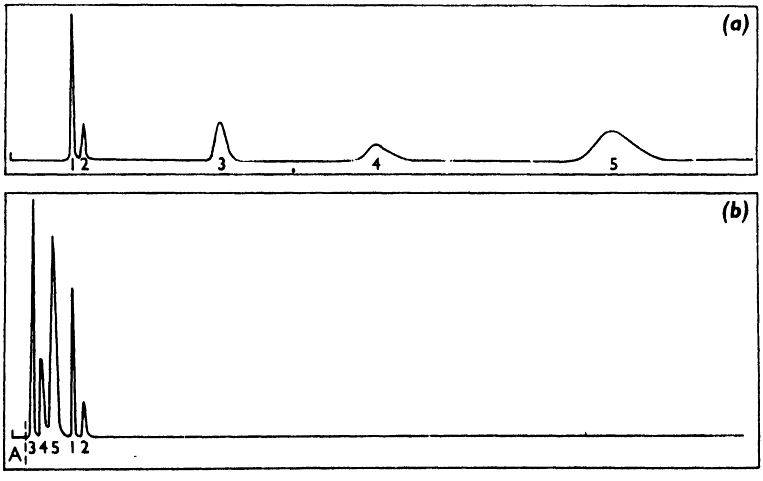


Fig. 8. Effect of reversing column order: (*a*) all peaks through column 1 then column 2; (*b*) peaks 3, 4 and 5 through column 1 only, peaks 1 and 2 through column 1 then column 2 then column 1 again (columns reversed at point A)

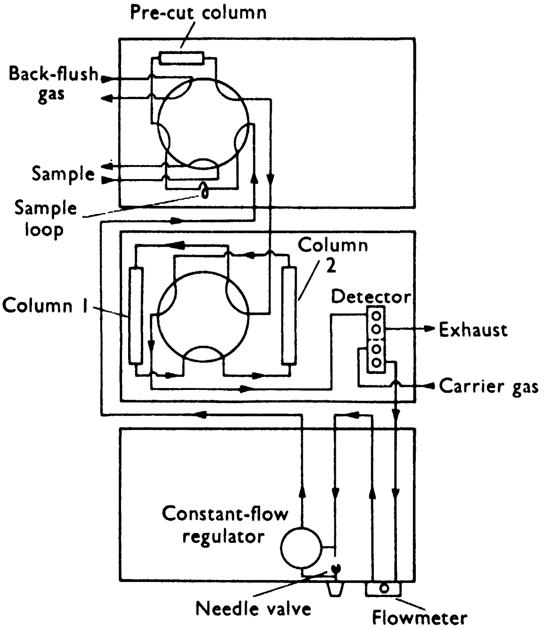


Fig. 9. Arrangement for removal of water and materials having high boiling-points, and reversal of column order

Fig. 10 shows an arrangement of two oven modules and two control modules for analysing materials of high and low boiling-points in one sample. In this particular instance no pre-cutting and back-flushing columns are required.

Fig. 11 shows the most complicated arrangement yet envisaged. In this example parallel samples are taken at a temperature above the dew-point of the sample. The samples are then transferred (without condensation) to parallel pre-cutting and back-flushing columns at a different temperature. Finally, the determinations of high and low boiling-point materials are carried out on two columns at two different temperatures. Figs. 12 and 13 are photographs of the arrangement shown in Fig. 11.

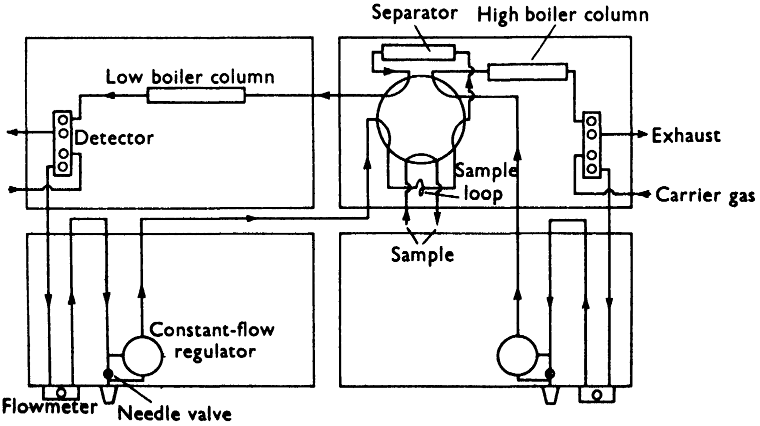


Fig. 10. Arrangement for the analysis of materials of high and low boiling-points on one sample

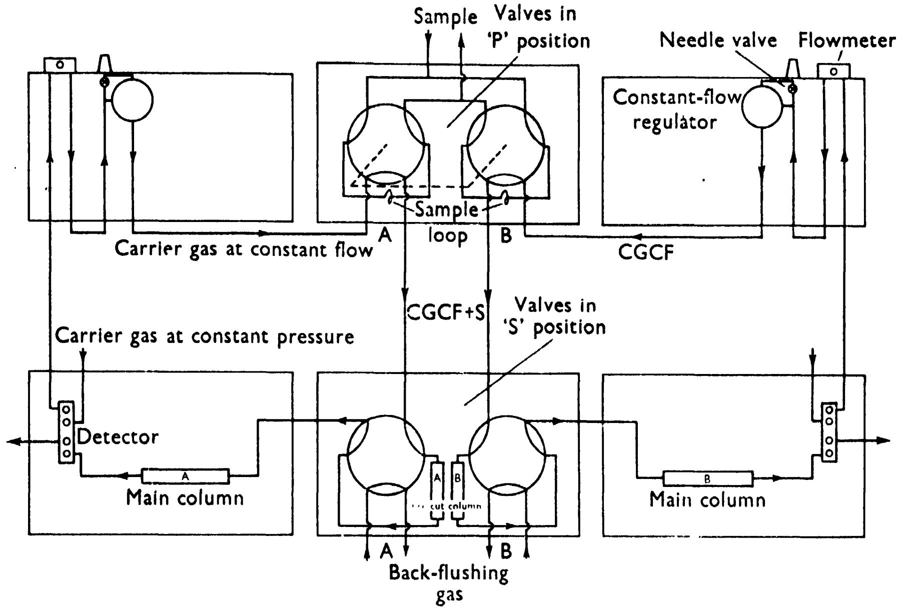


Fig. 11. Arrangement for parallel sampling for separate analysis of materials of high and low boiling-points with removal of water

PROBLEMS IN OPERATION

It is most important that the sample be kept in a single phase from the point of sampling to the point of exit from the modules. The entire sample line has, therefore, to be heated, and the bulkheads where the sample line enters the oven module must also be kept above the dew-point. The temperature of the sample loop should preferably be 20° C above the dew-point of the sample.

The successful operation of the system depends on the elimination of all gas leaks. Initially, difficulties were experienced with valve leakages, but, provided that the valves are polished to the necessary degree of flatness and lubricated with the recommended mixture, this problem has been overcome.

Experience has shown in fact that it is possible to analyse vapour samples of complex mixtures at temperatures up to 200° C.

USE OF THE EQUIPMENT

The equipment is intended primarily for the analysis of the products from laboratory reactors, but it can also be used for the preliminary application study required if the use of process analysers is being considered.

The system can also be used to obtain information about the dynamic behaviour of chemical reactors.

The apparatus is not flameproof and is not designed to be, and it is not intended to be used as a process analyser.

At present the maximum operating temperature that can be used is 200° C. This permits the analysis of a wide range of mixtures including mixtures of C_1 to C_4 hydrocarbons mixed with steam, air, product gases and such oxygenated products as aldehydes, ketones, acids and esters.

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SHORT PAPERS

Calcium Chloride Starch-dispersing Media

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THE Clendenning method[1] for determining starch polarimetrically is widely used and of general application. Its successful use depends on control of: (*a*) the pH of the medium, (*b*) the temperature and (*c*) the time of heating. A liquor with pH above 2·5 will produce too viscous a dispersion for ease of filtration (invariably required after de-proteination). Lower values of pH give lower values for the specific rotation and cause more marked degradation of the starch. Control at pH 2·3 appears optimum. This can be more easily effected by making up the medium according to Fraser, Brandon-Bravo and Holmes,[2] when it will be found that the change of pH on addition of acetic acid will be more gradual and easier to control. It is, however, open to objection to employ the usual calomel - glass electrode system to measure the pH of such a strong salt solution.

This led to some experiments to study the effect of various concentrations and types of acid, and also the mode of addition in order to arrive at a simple recipe that would ensure a good practical medium without the necessity always to refer to pH. The results obtained on acidification of the medium with acetic acid are shown in Fig. 1. With the medium usually recommended (sp. gr. 1·31) the change of pH is shown to be extremely rapid around 2·3. This is not so marked with calcium chloride solutions of specific gravity of 1·25 or less.

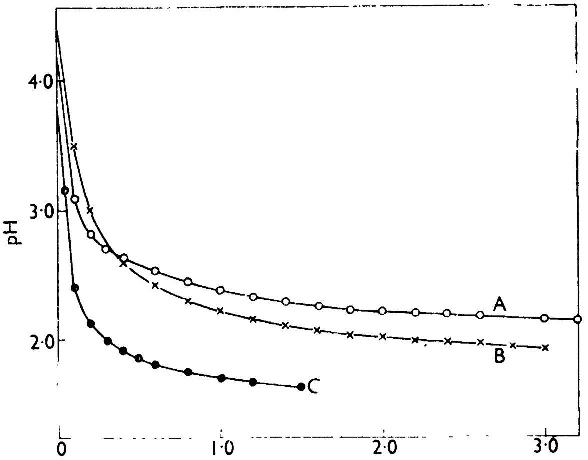

Fig. 1. Titration with 1 per cent. acetic acid of 50 ml of calcium chloride solution of different concentrations: curve A, sp.gr. 1·20; curve B, sp.gr. 1·25; curve C, sp.gr. 1·31

When a solution of calcium chloride containing sodium acetate is acidified with acetic acid the change in pH is much slower, and it would be feasible to make up a satisfactory medium empirically without necessarily referring to the pH (see Fig. 2).

The effect of controlling the acidity of the calcium chloride dispersing medium by means of boric acid instead of acetic acid was tried. From Fig. 3 it can be seen that a medium of desired acidity is simple to prepare without reference to pH. This medium proved a successful dispersing agent. It took longer, however, to disperse starch than did the calcium chloride - sodium acetate - acetic acid medium.

It is interesting to note that the pH obtained by using boric acid depends on the specific gravity of the calcium chloride solution, but this seems to have little effect on the dispersing power of the medium, provided that a certain concentration of boric acid is present in the medium. However, we recommend the use of the medium of specific gravity 1·34 and hence pH about 2·3.

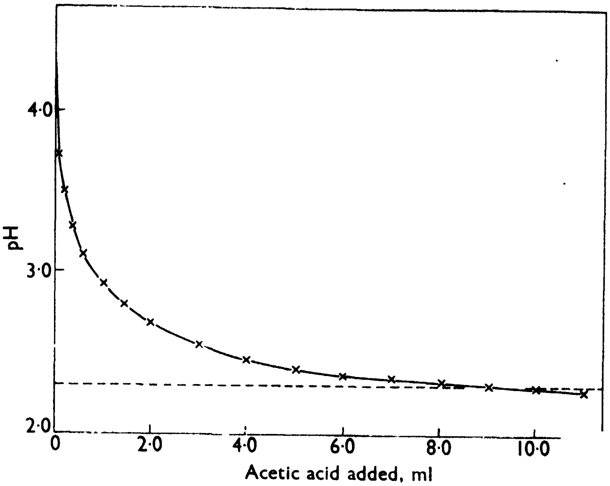

Fig. 2. Titration of 50 ml of calcium chloride - sodium acetate solution with 5 per cent. acetic acid

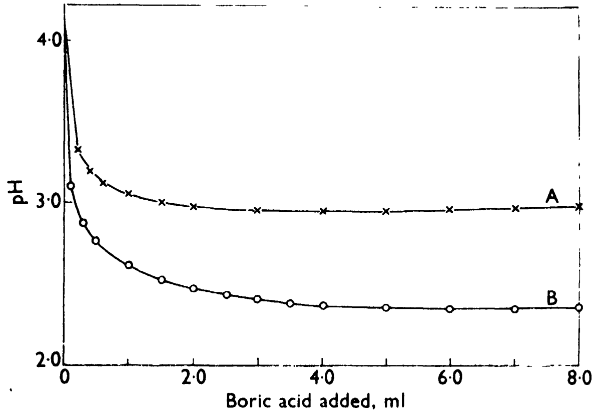

Fig. 3. Titration with 4 per cent. boric acid of 50 ml of calcium chloride solution of different concentrations: curve A, sp.gr. 1·31; curve B, sp.gr. 1·36

Other acids were also tried, *viz.*, benzoic, salicylic, citric and oxalic acids, to see if they could also be used to acidify the medium without referring to the pH, but none was successful.

The difficulty of controlling the temperature and time of heating required for the dispersion was overcome by using a bath of hot glycerol that was stirred mechanically and maintained at a steady temperature. The sample could then be heated for a definite time at a certain temperature, and after having been removed from the glycerol bath the sample was cooled in a water bath.

In order to check whether the direct polarimeter reading can be attributed to starch alone, precipitation with iodine - potassium iodide solution can be carried out. This operation effects an almost complete separation of starch from other polysaccharides likely to be present. The final reading of the recovered starch dispersed in sodium thiosulphate solution as described should be within the range of $R/2$ to $1{\cdot}04R/2$, where R is the primary reading.

METHOD

DISPERSING MEDIA—

Calcium chloride - sodium acetate - acetic acid medium—Dissolve 620 g of calcium chloride hexahydrate in 180 ml of water, filter the solution until it is clear, and adjust the specific gravity to 1·30. Add sodium acetate trihydrate (1 g per 100 ml of solution), and warm the solution to dissolve the sodium acetate. Adjust the pH to 2·3 by adding 10 per cent. acetic acid solution.

Calcium chloride - boric acid medium—Dissolve 620 g of calcium chloride hexahydrate in 150 ml of water, and filter the solution until it is clear. Add 10 ml of 4 per cent. boric acid solution for every 100 ml of solution, and adjust the specific gravity of the solution to 1·34 by adding distilled water.

REAGENTS—

Carrez's solution I—Dissolve 21·9 g of zinc acetate dihydrate and 3 ml of glacial acetic acid in water, and dilute to 100 ml.

Carrez's solution II—Dissolve 10·6 g of potassium ferrocyanide in water, and dilute to 100 ml.

Iodine - potassium iodide solution—Dissolve 30 g of iodine and 50 g of potassium iodide in water, and dilute to 250 ml.

PROCEDURE—

Mix 2·5 g of starch to a smooth paste with 10 ml of water, and add 50 ml of either of the calcium chloride media. Heat the mixture in a glycerol-bath at 116° to 118° C for 25 minutes when calcium chloride - sodium acetate - acetic acid is the dispersing medium used, or for 50 minutes when the calcium chloride - boric acid medium is used. Stir the mixture occasionally during this period. Cool the mixture in a water bath, transfer it to a 100-ml graduated flask, and make up to 90 ml with calcium chloride solution. Add 2 ml of Carrez's solution I, mix, and then add 2 ml of Carrez's solution II. Make the contents of the flask up to 100 ml with calcium chloride solution at 20° C, shake, and filter through a Whatman No. 541 filter-paper. Reject the first 10 ml of filtrate. Determine the optical rotation of the remainder of the filtrate in a 20-cm polarimeter tube at 20° C. Calculate the percentage of starch in the solution from the equation—

$$\text{Percentage of starch} = \frac{P \times 10^4}{2 \times W \times 203}$$

where P is the polarimeter reading in degrees and W is the weight, in grams, of starch taken.

RECOVERY AS A STARCH - IODINE COMPLEX—

By pipette transfer 25 ml of the filtrate to a 100-ml centrifuge tube, and add 20 ml of 20 per cent sodium chloride solution and then 2 ml of the iodine - potassium iodide solution. Add sodium chloride solution until the tube is about two-thirds full, stopper the tube, and shake gently. Then wash particles adhering to the stopper into the tube with water. At least 10 minutes after the start of precipitation, spin the tube in a centrifuge at 3000 r.p.m. for 10 minutes. Decant the supernatant liquid, wash the precipitate with sodium chloride solution, and again spin in the centrifuge. Suspend the precipitate in 10 ml of sodium chloride solution, and decompose by adding a few millilitres (the minimum amount) of 12·5 per cent. sodium thiosulphate solution, with stirring. Transfer the solution to a 50-ml calibrated flask, and make up to the mark with sodium chloride solution. Determine the optical rotation in a 20-cm polarimeter tube at 20° C.

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Titration of Water in Plasma or Red Cells with Karl Fischer Reagent

BY J. D. PRYCE

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THE determination of water by titration with Karl Fischer reagent is now a commonplace procedure. The usual practice is to employ a potentiometric technique. The visual end-point originally described is unsatisfactory, since it involves a colour change from yellow to brown. An alternative visual method is described here, for the benefit of those who lack the potentiometric apparatus.

During the reaction between Karl Fischer reagent and water, sulphur dioxide present in the reagent is consumed. In the absence of sulphur dioxide, Malachite green shows a strong green colour; this disappears when free sulphur dioxide is present.

METHOD

APPARATUS—

An ordinary 25-ml burette is used, protected from the atmosphere by a calcium chloride reservoir; a bored rubber bung is placed over the tip of the burette and a 50-ml Erlenmeyer flask is fitted on to the bung. Specimens must be stored in stoppered containers, and all pipettes or oral suction tubing should be lightly plugged with cotton-wool, to minimise contamination from expired vapour.

EAGENTS—

Karl Fischer reagent—As supplied by Hopkin & Williams Ltd.

Malachite green stock solution—Dissolve 0·5 g of the dye in 100 ml of dry methanol.

Malachite green working solution—Add 2·5 ml of the stock solution to 1 litre of dry methanol.

Red cell diluent—Add 45 ml of analytical-reagent grade butane-1,3-diol to 55 ml of dry methanol.

PROCEDURE—

Rinse the apparatus with dry methanol before use, and fill the burette with Karl Fischer reagent. By pipette place 10 ml of working solution in the flask, and titrate with Karl Fischer reagent to the end-point. The colour changes are from blue-green to emerald green, olive green and finally to reddish brown. The red-brown colour at the end-point is easily seen through the side of the flask by using a bench-light behind it, but the colour is not readily visible from above. If the reagent is in good condition, one drop or less is sufficient at the end-point; the red-brown colour persists for at least a few minutes. The reaction is not instantaneous, so a few seconds must be allowed after each addition when the titration is nearing the end-point. After titrating the blank solution, remove the flask, and rinse 0·02 ml of water into the solution. Then continue the titration to the end-point. During the titration loosely hold the neck of the flask against the bung, and swirl the flask continuously.

Determination of plasma water—Having standardised the reagent, titrate a fresh 10-ml portion of working solution to the end-point; then rinse 0·02 ml of plasma into the flask, stopper the flask, and swirl vigorously to extract the water. Then titrate as described above.

Determination of red cell water—Rinse two stoppered centrifuge tubes with dry methanol, and drain; place 5 ml of red cell diluent in each of the two tubes. Rinse a 0·1-ml pipette with the red cell diluent, and blow out the residue. Draw the packed red cells up to the mark, and rinse them into one of the two tubes. Stopper the tubes, and shake for 30 seconds; remove the stoppers, and spin the tubes in a centrifuge (radius 14 cm) for 1 minute at 1000 r.p.m. Remove 2 ml of the water extract, and titrate as described for plasma.

CALCULATION—

For plasma, the relation of the two titrations is one of simple proportionality. For red cells it is necessary to apply a correction factor. If the pipette is not initially wetted with the diluent, the packed cells tend to coagulate in the pipette when it is rinsed out. However, owing to the

TABLE I

COMPARISON OF GRAVIMETRIC AND TITRIMETRIC DETERMINATIONS ON THE SAME SPECIMENS

Water found in plasma by—		Water found in red cells by—	
gravimetric method, ml per 100 ml	titrimetric method, ml per 100 ml	gravimetric method, ml per 100 ml	titrimetric method, ml per 100 ml
93·1	92·6	75·8	74·9
93·1	92·1	76·6	75·6
93·3	91·8	75·2	76·1
92·9	91·8	77·8	76·9
92·7	92·6	74·2	73·4
92·9	91·8	75·0	74·4
		72·0	72·4

viscosity of the diluent, the effective volume of the pipette is reduced by wetting. The effective volume was measured by a colorimetric technique and also by weighing on a micro balance, and both methods showed it to be 94 per cent of its nominal value. This factor must therefore be included in the calculation.

COMPARISON WITH GRAVIMETRIC DETERMINATION

The accuracy of the procedures both for red cells and plasma was assessed by comparing the results obtained with those obtained by drying approximately 2-g samples to constant weight. The gravimetric values obtained were converted to ml per 100 ml by measuring the specific gravity of the samples. The results are shown in Table I. The standard error of the method for plasma was ±1·1 per cent., and for red cells ±2·3 per cent.

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The Determination of Cysteine, Thioglycollic Acid, Cyanide and Dithioglycollic Acid

By M. WROŃSKI

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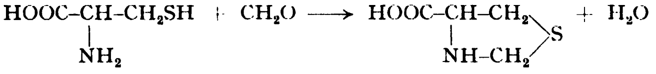
THE kinetic investigation of the reactivity of the disulphide bond in cystine and other organic disulphides requires the application of simple and rapid methods for determining, in the presence of each other, both the initial compounds and the products of the reaction. Some of the analytical procedures that have been successfully applied in kinetic study are summarised here.

The method for determining cysteine and thioglycollic acid in the presence of each other is based on the fact that formaldehyde reacts readily with cysteine, according to the equation—



$$\begin{array}{l} HOOC\text{-}CH\text{-}CH_2SH \\ \quad\;\; | \\ \quad\; NH_2 \end{array} + CH_2O \longrightarrow \begin{array}{l} HOOC\text{-}CH\text{-}CH_2 \diagdown \\ \quad\;\; | \qquad\qquad\;\; S \\ \quad\; NH\text{-}CH_2 \diagup \end{array} + H_2O$$

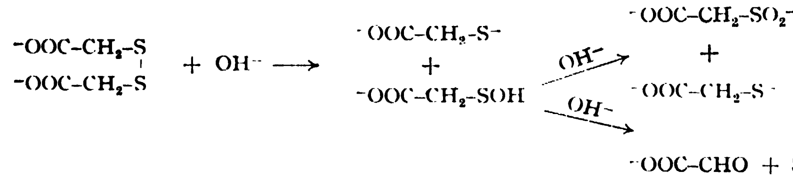
and that the compound formed does not react with *o*-hydroxymercuribenzoic acid. On the other hand, thioglycollic acid and other mercaptans that have no primary or secondary amino-group can be titrated with *o*-hydroxymercuribenzoic acid in the presence of formaldehyde without interference.

Cyanide and mercaptans having no amino-group can be determined in the presence of each other by the method briefly described below. One sample of the mixture is treated with acrylonitrile[1] to remove the mercaptan according to the equation—

$$RSH + CH_2{=}CHCN \longrightarrow RS\text{-}CH_2\text{-}CH_2CN$$

and the cyanide is then titrated with silver nitrate solution.[2] A second sample of the mixture is treated with formaldehyde to remove the cyanide, and the mercaptan is titrated with *o*-hydroxymercuribenzoic acid.[3] For cysteine the formaldehyde must be replaced by an amount of silver nitrate equivalent to the cyanide content.

The determination of dithioglycollic acid is based on its rapid decomposition in boiling alkaline solution, according to the equation—



$$\begin{array}{l} {}^{-}OOC\text{-}CH_2\text{-}S \\ \qquad\qquad\quad | \\ {}^{-}OOC\text{-}CH_2\text{-}S \end{array} + OH^{-} \longrightarrow \begin{array}{c} {}^{-}OOC\text{-}CH_2\text{-}S^{-} \\ + \\ {}^{-}OOC\text{-}CH_2\text{-}SOH \end{array} \begin{array}{l} \xrightarrow{OH^{-}} \begin{array}{c} {}^{-}OOC\text{-}CH_2\text{-}SO_2^{-} \\ + \\ {}^{-}OOC\text{-}CH_2\text{-}S^{-} \end{array} \\ \xrightarrow{OH^{-}} {}^{-}OOC\text{-}CHO + S^{2-} \end{array}$$

Although the thioglycollic acid and sulphide ions are not produced in equivalent amounts, the dithioglycollic acid content can be calculated from the equation—

$$\left[\begin{array}{l} HOOC\text{-}CH_2\text{-}S \\ \qquad\qquad\quad\; | \\ HOOC\text{-}CH_2\text{-}S \end{array}\right] = [S^{2-}] + \frac{[{}^{-}OOC\text{-}CH_2S^{-}] - [S^{2-}]}{1{\cdot}5} \qquad \text{.. .. ..} \quad (1)$$

if the thioglycollic acid and sulphide are determined separately. If the original solution contained sulphide and mercaptans, only those amounts produced by the decomposition must be taken into account.

METHOD FOR DETERMINING CYSTEINE AND THIOGLYCOLLIC ACID

PROCEDURE FOR CYSTEINE PLUS THIOGLYCOLLIC ACID—

To a 25-ml sample add 1 ml of N sodium hydroxide, and titrate with 0·005 to 0·05 N *o*-hydroxymercuribenzoic acid, with thiofluorescein as indicator, until the blue colour sharply disappears.

PROCEDURE FOR THIOGLYCOLLIC ACID—

To a 25-ml sample add 1 ml of 37 per cent. w/v formaldehyde and 1 ml of N sodium hydroxide, set aside for 3 to 5 minutes at 22° C, and then titrate with 0·005 N *o*-hydroxymercuribenzoic acid in the presence of thiofluorescein.

Some of the results obtained by this method are shown in Table I. Note that the presence of cystine and dithioglycollic acid does not interfere in either titration.

METHOD FOR DETERMINING CYSTEINE AND CYANIDE

PROCEDURE FOR CYANIDE—

To a 40-ml sample of the mixture add 5 ml of N sodium hydroxide and 2 ml of a M solution of acrylonitrile in water, set aside for 5 to 15 minutes at 22° C, and then titrate the cyanide with 0·01 N silver nitrate, with thiofluorescein as indicator.

PROCEDURE FOR CYSTEINE—

To a 40-ml sample of the mixture add 5 ml of N sodium hydroxide and the same amount of 0·01 N silver nitrate as was required to complete the previous titration (see "Procedure for Cyanide"). Titrate the cysteine with 0·005 N *o*-hydroxymercuribenzoic acid, with thiofluorescein as indicator.

Note that the presence of cystine and organic thiocyanates does not interfere in either titration.

TABLE I

DETERMINATION OF CYSTEINE AND THIOGLYCOLLIC ACID

Cysteine present, mmoles	Thioglycollic acid present, mmoles	Cysteine found, mmoles	Thioglycollic acid found, mmoles
0·0000	0·0835	0·0005	0·0830
0·1610	0·0000	0·1600	0·0010
0·0962	0·0278	0·0960	0·0283
0·0643	0·0556	0·0646	0·0556
0·0321	0·0835	0·0318	0·0840
0·0321	0·0278	0·0316	0·0282
0·0642	0·0278	0·0640	0·0283
0·0321	0·0556	0·0320	0·0550

METHOD FOR DETERMINING DITHIOGLYCOLLIC ACID

PROCEDURE—

Prepare a solution of the sample in sodium hydroxide. Adjust the concentration of the sodium hydroxide to 0·5 N, heat the solution to boiling, and boil for about 20 seconds. Cool the solution, add sufficient 5 N ammonium sulphate to give a slight excess (in respect of the amount of sodium hydroxide present), transfer to 100-ml calibrated flask, and dilute to the mark with water.

Take a 40-ml portion of this solution, add 1 ml of M acrylonitrile solution, set aside for 5 minutes at 22° C, and then determine the sulphide content by titrating with 0·005 N *o*-hydroxymercuribenzoic acid, with thiofluorescein as indicator.

Take a second 40-ml portion of the solution, and determine the thioglycollic acid *plus* sulphide with 0·005 N *o*-hydroxymercuribenzoic acid, with thiofluorescein as indicator.[1]

To verify the accuracy of the method, 10-, 20-, 30-, 40- and 50-ml portions of a 0·00173 M solution of dithioglycollic acid were taken and analysed by the procedure described above. The concentration of dithioglycollic acid in each portion was calculated from equation 1; the results were 0·00169, 0·00172, 0·00168, 0·00176 and 0·00176 M, average 0·00172 M.

After the decomposition of the dithioglycollic acid with alkali, the ratio of sulphide to thioglycollic acid in the resulting solution is in the range 0·75 to 0·83.

REFERENCES

1. Wroński, M., *Analyst*, 1960, **85**, 526.
2. ——, *Ibid.*, 1959, **84**, 668.
3. ——, *Chem. Anal., Warsaw*, 1961, **6**, 859.

Received *December 27th*, 1962

Book Reviews

ULTRAPURIFICATION OF SEMICONDUCTOR MATERIALS. Proceedings of the Conference on Ultrapurification of Semiconductor Materials, Boston, Massachusetts, April 11–13, 1961. Edited by M. S. BROOKS and J. K. KENNEDY. Pp. xiv + 655. New York and London: The Macmillan Company, a Division of The Crowell-Collier Publishing Company. 1962. Price $12.50; 94s.

This book is a record of the proceedings of the Conference on Ultrapurification of Semiconductor Materials that took place at Boston, Massachusetts, in April, 1961. The Conference was sponsored by the Electronics Research Directorate, Air Force Cambridge Research Laboratories, Bedford, Mass., and among the papers contributed a number were from this country, France, Germany and Japan.

There are three main sections to the book: chemical preparation and purification of semiconductor materials; the detection of trace impurities in semiconductor materials; physical preparation, purification and measurements of semiconductor materials. Section II on the detection of trace impurities occupies 200 pages and should be read by every analyst who is concerned to keep abreast of developments in technique. In Parts I and III some indication is given of the phenomenal purity that has been achieved in materials used in this special field. Germanium and silicon are possibly the purest elements available in the world. The chemical and physical methods employed to attain these high purities have been adapted with advantage to other fields of interest, including organic chemicals and metals. The nature if not the magnitude of the analytical problems created by these developments is self-evident, and few of them have been satisfactorily solved. This is not to say that little progress has been made, indeed, where the concentrations of impurity are in the range above 1 part per million it is considered that the analyst has no particular problem—"emission spectroscopy, colorimetry and other wet chemical methods have been more than adequate"! The interest now lies in the range of parts per (American) billion and even lower.

The three main techniques discussed in the various papers are activation analysis, emission spectroscopy and spark source mass spectrometry. In the first paper in Part II, G. H. Morrison discusses in general terms the merits and demerits of these three main approaches, and he makes reference also to a number of the other and more novel techniques that have been explored. Activation analysis is of great value, but Morrison rightly draws attention, for example, to the time-consuming radiochemical separations that are sometimes required. The inability to determine by this technique some of the elements that are of major interest also limits its value. In emission spectroscopy progress, which has been considerable, has involved the development of pre-concentration techniques and of improvements in the techniques of volatilisation and of excitation.

In the discussion and in the various papers on solid source mass spectrometry emphasis is laid on the need for standards and on the relatively slow nature of the operations; not more than two samples per day can be readily accommodated.

Morrison offers some general assessment of the relative value of the three techniques and emphasises the need for new and highly specialised methods to deal with some of the special problems that arise in connection with the technology of semiconductors. Some novel techniques were in fact presented to the Conference. Andrychuk and Jones of Texas Instruments contributed a paper entitled "Electron Gun Type Excitation Source for Spectrographic Determination of O_2, N_2, H_2, P and S and Halogens in Gallium Arsenide." This method of excitation was introduced in order to circumvent the difficulties of more conventional techniques. Unfortunately, the sensitivity obtained was inadequate for most semiconductor analysis, but the technique should justifiably receive further study and development.

In another paper the "Hall Effect" is considered as an analytical tool in ultrapure silicon and germanium. It is known that the value of the Hall coefficient at room temperature in germanium or silicon can be related to the net concentration of active centres and that measurements as a function of temperature may give indications of the total number of such centres. Readers will need to refer to the conclusions of this paper to enable them to judge the degree of success likely to be achieved by this approach.

Other novel approaches include: "Space-charge Current Measurements as a Technique for the Detection of Trace Impurities in Ultrapure Material"; "Trace Analysis in Semiconductor

Crystals by X-ray Diffraction Microscopy"; "Determination of Small Stoichiometric Deviations in Oxide Monocrystals."

It may be fairly said the the section of this book dealing with analysis does more to expose the limitations and shortcomings of analysis in this new field of semiconductor materials than to demonstrate its successes. Nevertheless, it is not without value for the challenge to the analyst is made clear, not only to modify and improve his techniques so as to cope with the determination of many elements at levels of much less than one part per million, but also to give earnest consideration to new kinds of measurement of new kinds of physical entities that might properly be considered to come within the needs and the province of contemporary analytical information.

R. C. CHIRNSIDE

PROGRESS IN NUCLEAR ENERGY. Series IX. ANALYTICAL CHEMISTRY. Volume 3. Parts 1–3. Edited by CARL E. CROUTHAMEL. Pp. vi + 93. Oxford, London, New York and Paris: Pergamon Press. 1962. Price 30s.

This recent publication of Volume 3, 1–3, of the "Progress in Nuclear Energy, Series IX" consists of 93 pages enclosed in a limp, but robust, cover, and it will undoubtedly arouse nostalgic recollections in those fervent wielders of paper knives, who remember the uncut pages in the quality books of an earlier age. Some modernists may, however, regard the occasional uncut page with some irritation, and perhaps annoyance.

The three chapters presented on nuclear science are most informative and provide much information of considerable value to both the tyro and the research scientist.

T. T. Sugihara has written a comprehensive review of those radiochemical methods applicable to low-level determinations where the beta disintegrations may be less than 10 per minute and the signal-to-noise ratio approaches unity. He prefaces his account with a most useful appraisal of those considerations that affect the "blank determination," and illustrates the analytical techniques by reference to the analysis of ^{40}K in meteorites, cobalt in cyclotron targets, radionuclides from spontaneous fission, etc. A useful bibliography of references to specialist papers is supplemented by an accompanying tabular code covering 62 elements.

In the second chapter, B. Rider and C. Ruiz deal most lucidly with the complex problems associated with the assessment of the "burn up" of nuclear fuel in atomic reactors. The methods described involve (*a*) the determination of specified fission products, (*b*) the measurement before and after irradiation of the fuel isotope and (*c*) non-destructive measurements, *e.g.*, calorimetry. The general features of the analytical techniques are described in fair detail, and the theoretical discussions cover not only the depletion of ^{235}U, but consider also the burn up of the resulting plutonium, ^{239}Pu, and the effect of accompanying radionuclides.

This paper on the determination of the atom per cent. fission is most usefully illustrated with factual results and will be eagerly read by everyone with a deep scientific interest in those more complex problems that attend irradiation studies.

The laboratory problems associated with the handling of the actinide elements are succinctly described by M. Steindler in Chapter III. Hazards due to criticality considerations are not covered in this treatment, but the paper abounds with valuable data relating to the biological hazards of penetrating radiations. One question receiving comprehensive treatment is the fast-neutron flux hazard associated with many actinide elements because of (α,n) reactions, resulting from the presence of light element impurities.

Precautions essential to the safe handling of the isotopes of thorium, uranium, plutonium, americium, etc., are clearly described, and attention is drawn to the complicating biological effects caused by the presence of residual daughter radionuclides or of isotopic transuranic elements. Much useful numerical data is presented in columnar form, and the information presented will be of the greatest value to scientists who have to design laboratories and equipment for actinide studies. Engineers, chemists and physicists alike will welcome this volume with its authoritative treatments of topics of the greatest importance to those who work in the ever-extending fields of nuclear science and technology.

D. T. LEWIS

GERMAN-ENGLISH CHEMICAL TERMINOLOGY: AN INTRODUCTION TO CHEMISTRY IN ENGLISH AND GERMAN. By ALEXANDER KING, C.B.E., D.Sc., A.R.C.S., D.I.C., and Prof. Dr. HANS FROMHERZ. Fourth Edition. Pp. xx + 588. Germany: Verlag Chemie. 1963. Price DM 48; $12.00.

German holds an unchallenged position as the second language for the English chemist, both the research chemist and the industrial chemist, and, though there may now be arising a challenger in Russian, it is not likely that German will be displaced. It is not an easy language to learn, particularly if, as generally happens with the chemist, it is self-taught. True there have been a number of books on "Chemical German," but they have usually been a kind of anthology of the classical chemical papers in German, so that the student is often baffled by peculiarities of syntax and idiom and is frequently left in some doubt as to the accuracy of his translation. This volume should remove any such doubt in addition to providing enlightenment on sentence formation. Though sub-titled "An Introduction to Chemistry in English and German," it is, rather, a fascinating device to introduce to both English and German chemists the scientific and technical terms of their profession in their native contexts. This is achieved by printing English and German texts on facing pages in which the corresponding terms are picked out in italics, with almost a line for line correspondence of the text in both languages.

From the chemical point of view, it might be entitled "An Outline of Chemistry," since the text covers the whole field in 225 pages of each language. The way in which this has been done to introduce ten thousand terms, from purity to parity, in their natural habitat, is a real *tour de force*.

The work is divided into five major parts—the foundations of chemistry (72 pp.), inorganic (92 pp.), organic (83 pp.) and physical (120 pp.) chemistry and the structure of matter (72 pp.). The first of these parts ranges from the classification of matter and crystallography to the classification of the elements and does not overlook the laboratory with its tools and crafts nor the library. The second part deals with the common non-metallic elements and metals, some major industrial products and analysis, and part three covers aliphatic and aromatic chemistry and biochemistry. Physical chemistry claims most space and deals with thermodynamics, surface chemistry, kinetics and electro- and photo-chemistry. The last part includes atomic structure, valency, with molecular orbitals and ligand theory, and nuclear chemistry.

The work is remarkably up to date; in testing its vocabulary with terms from recent publications, the only one I failed to find was HETP. The last two parts, in particular, are useful reference works. To get the fullest value from such a work, a good index is essential and two such indexes have been provided, one in each language. Not only do the indexes refer one to the page, but also to a 5-line section of the page. There are also useful appendixes of English and German abbreviations, of mathematical terms and of symbols of the International Phonetic Association, used to give the pronunciation of some English terms.

This is by far the most helpful volume I have met to assist the English chemist to read and understand German chemical papers. This fact is obviously widely appreciated, since this is the fourth edition of the work, which first appeared in 1934 with four thousand terms. It would have saved me much brain-racking had I encountered it then. J. I. M. JONES

THIN FILM CHROMATOGRAPHY. By E. VERNON TRUTER, Ph.D., B.Sc., A.R.C.S., D.I.C. Pp. xii + 205. London: Cleaver-Hume Press Ltd. 1963. Price 37s. 6d.

There is no doubt that thin-film chromatography has provided a major advance in the task of separating chemical substances one from another. It supplements its forerunners in chromatographic methods. The technique is elegant, simple to apply, quick in giving information, and sparing of working material. It impresses all who try it. It is specially useful for studying weakly polar materials such as lipids, and it has proved a great addition to our repertory of methods for attacking the difficult problems involved in separating organic compounds generally. Thus thin-film chromatography will give good separations of amines, amino acids, drugs, indole derivatives and metabolites, insecticides, lipids, nucleotides, nucleosides and nucleic bases, vitamins, organo-metallic compounds and inorganic ions. It is available for straight adsorption chromatography, partition and reversed-phase chromatography and indeed any of the methods associated with paper and column chromatography.

This book presents one of the first—if not the first—connected account of the possibilities and uses of thin-film chromatography. It is divided into three sections. In the first the author describes the principles and practice of straightforward qualitative analysis by its means; in the second he deals with special techniques for resolving what he calls "difficult mixtures," with quantitative analyses and with small-scale preparations; and in the last section he reviews the literature up to the end of 1962.

He prefaces the book with a graph showing how interest has grown in the subject, and how it grows ever faster. There seems to be a special appeal to the methodically-minded German race, for the graph shows that it is largely their contribution that has rocketed the number of papers published lately.

The author is to be congratulated on a clear exposition of his subject and on the comprehensive nature of his book. His style is succinct and to the point, and he is easy to follow and copy. He illustrates a good deal of the apparatus now commercially available for thin-film chromatography, and refers to more. Intending users of the process should not be put off by its apparently high price; you will find that you can do all you want to with apparatus made on lines similar to those described and built in your own laboratory. K. A. WILLIAMS

GAS CHROMATOGRAPHY 1962. Edited by M. VAN SWAAY. Pp. lii + 411. London: Butterworth & Co. (Publishers) Ltd. 1962. Price 100s.

This book is a collection of all the proceedings of the fourth international symposium on gas chromatography. The symposium was organised jointly by the Fachgruppe Analytische Chemie of the Gesellschaft Deutscher Chemiker and the Gas Chromatography Discussion Group of the Hydrocarbon Research Group of the Institute of Petroleum and held at the Auditorium Maximum, Hamburg, June 13th to 16th 1962.

It contains twenty-seven papers presented at the symposium, verbatim reports of the discussions on these papers and also reports of the three Panel Discussions on selected aspects of gas chromatography. The Panel Discussions, which dealt with (*a*) large-scale gas chromatography, (*b*) quantitative aspects of gas chromatography and (*c*) qualitative aspects of gas chromatography, constituted an innovation that I hope will be repeated at any future symposia of this nature. The papers are classified in three sections, as on earlier occasions.

Section I deals with the theory of gas chromatography, and some papers give mathematical treatments of the behaviour of capillary and other columns and discuss the use of gas chromatography as a method of determining adsorption values and other physical constants. There are two papers in this Section that I commend to the analyst. The first, by Desty and his colleagues, challenges two of the "axioms" of gas chromatography, *viz.*, that relative retention times are independent of the nature of the carrier gas and of the column pressure. The other discusses a promising series of polar liquids for use at temperatures between 150° and 250° C for which there is a great demand. Further, as the liquids consist of cyaroalkyl-substituted siloxane chains there is a possibility of adjusting the degree of polarity by controlling the number of substituent groups in the chain.

Section II is headed "Apparatus and Technique." It contains papers on some promising new techniques, such as programmed temperature operation from sub-ambient temperatures to 300° C or over, the use of narrow packed columns, and use of mass spectrometry in conjunction with gas chromatography. Some papers in this section are perhaps more appropriate to Section I and of interest to the theoretical physical chemist, if not particularly helpful to the analyst. There are two interesting papers for the industrial chemist dealing with a continuous counter-current separation procedure based on gas chromatography and with the use of gas chromatography for automatic process control.

Section III, entitled "Applications," contains some interesting papers on the pre-treatment of samples before gas chromatography, *e.g.*, thermal cracking of hydrocarbons, and preparation of derivatives of phenols and fatty acids. Trace analysis is represented and also the analysis of materials less frequently met with hitherto in gas chromatography, such as inorganic gases and compounds.

Each paper has its own bibliography, and Dr. M. Van Swaay has done an excellent job in producing a book that contains something to interest all gas chromatographers, whether they are primarily analysts or not. B. A. ROSE

GAS CHROMATOGRAPHY. By HOWARD PURNELL. Pp. viii + 441. New York and London: John Wiley & Sons Inc. 1962. Price 90s.

According to the dust cover the author's aim has been to provide the novice, having no previous experience of physical chemistry, with sufficient theory and practical data to construct a first-class apparatus and solve any analytical problem with minimum experimental effort. The author's preface indicates a considerably modified ambition and intending purchasers are recommended to ignore the publisher's summary.

A brief introduction is followed by four chapters on the physico-chemical background of gas chromatography. Theoretical principles and equations are discussed for the processes of solution, adsorption and partition, diffusion in gases and liquids and the flow of gases. Although the treatment is largely theoretical and considers ideal conditions, every possible effort is made to embrace the mechanisms of gas-chromatographic processes. The treatment has suffered from the necessity of condensing a comparatively wide field into 70 pages. The reader who is renewing acquaintance with subject matter studied some years ago may well find this section heavy going, particularly since he is referred to alternative sources of information on perhaps too many occasions.

Chromatographic theory is discussed in the next section of 160 pages with chapters on techniques, the theoretical plate concept, rate theories, experimental test of rate theories and thermodynamics of gas chromatography. The subject matter is pursued in far greater theoretical detail than is found in more recent books on the subject. Theory is supported by detailed accounts of extensive practical studies carried out in the author's laboratories, particularly on the subjects of support materials and stationary phases. In a text-book aiming at theoretical explanation of every facet it was refreshing and perhaps consoling to read that choice of stationary phase is often a matter of trial and error.

The third section reviews gas-chromatographic practice with chapters on equipment and materials, detectors, analytical techniques and non-analytical applications. The coverage is adequate and general; no attempt has been made to indicate specific operating conditions for particular analysis. The author has written in depth about the more popular techniques of gas chromatography, particularly in those areas with which he is closely familiar. Although the preface is dated July, 1962, references suggest that the text was largely completed by mid-1960. It is perhaps significant that text-books on gas chromatography, particularly those suffering delays in publication, are more than usually prone to becoming dated by advances in the field. In this sense the author would now, most certainly, want to revise his predictions on the future of electron-capture techniques. In a section devoted to quantitative analysis the author discusses relative merits of peak height or area measurements, but no suggestion is made that area is also defined by strip chart speed and that this has an optimum value for good correlation between sample size and response.

This book will be well received by the physical chemist using gas-chromatographic techniques, particularly those undertaking research. The author has faced a major problem in the matter of presentation, and many will feel he has achieved the best possible compromise. The mathematical treatment in the early chapters may well deter the more mature commercial analyst. The latter would have profited from a preface that included a list of symbols of both modern physical chemical terminology and also those proposed by the I.U.P.A.C. and B.S.I. for gas chromatography. Such expressions as $\ln.f_1^o$, μ^o, $(V_g^n)^o$ would have caused less disturbance had the significance of the power factor been explained in a glossary. In addition, f would not have been used for both activity coefficient and gas compressibility factor (pressure gradient correction factor j).

Brief accounts are given of the electrical circuitry of katharometers, an impedance convertor system for flame ionisation detectors and the effect of high capacitance on the time constant of signal response. The design, construction and operation of voltage-control devices, amplifiers and recorders are not discussed.

A list of references is given at the end of each chapter, making a total of more than 630. The book was printed in the U.S.A. and appears to be priced accordingly. It is relatively free from printing errors. A limited index detracts from its general value as a reference book.

W. L. SHEPPARD

CHROMATOGRAPHY AND ELECTROPHORESIS ON PAPER: A TEACHING LEVEL MANUAL. By J. G. FEINBERG, B.Sc., M.Sc., D.V.M., M.I.Biol., and IVOR SMITH, B.Sc., Ph.D., F.R.I.C., M.I.Biol. Pp. xii + 130. London: Shandon Scientific Company Ltd. 1962. Price 21s.

Several books have been written on the theory and practice of both paper chromatography and electrophoresis. In general, these books are intended for the practising analyst rather than for the student of chemistry. The aim of the authors of this manual, however, has been to present information on these subjects in a manner suitable for teachers and students. For example, although carefully defining and explaining the meaning of R_F value, the authors have not thought it within the scope of this publication to include tables, but prefer to list a bibliography of more advanced works for the reader who intends to pursue the subject. Nevertheless, any analytical chemist who wishes to apply these techniques for the first time would benefit by reading this well laid-out volume.

It is divided into two sections, the first dealing with paper chromatography and the second with zone paper electrophoresis. The underlying principles of both techniques are clearly and simply explained, and an intelligent use has been made of diagrams. There is a full account of experimental practice in these fields, and fifteen experiments in paper chromatography and a further nine in electrophoresis are included. The experiments are sufficiently detailed to enable the student to carry them out without constant supervision and are designed to familiarise him with some of the more important principles and applications. The fact that this manual has been written in conjunction with the manufacturers of the proprietary equipment upon which the experiments are based does not detract from its general usefulness. D. M. PEAKE

THE ANALYTICAL CHEMISTRY OF INDIUM. By A. I. BUSEV. Translated from the Russian by J. T. GREAVES. Pp. xvi + 288. Oxford, London, New York and Paris: Pergamon Press. 1962. Price 84s.

Professor Busev has collated all the available information on the detection, separation and determination of indium, the major part of which has originated from Russian and Western literature published up to the beginning of 1957. The subject matter is presented mainly in the form of a review, but also contains many detailed analytical procedures that have proved reliable; the author's own recommendations and results with regard to reproducibility, sensitivity and accuracy are also appended.

The opening chapter reviews the chemistry of indium and the manner in which the chemical and physical properties of indium compounds form the basis of available analytical methods. Successive chapters examine methods of analysis in detail, according to the reaction of indium: under conditions of hydrolysis, with inorganic anions, halide and thiocyanate ions, carboxylic and amino-carboxylic acids, reagents containing hydroxyl groups, and with reagents containing sulphydryl groups. The final three chapters describe methods based on the reduction of tervalent indium (mainly polarographic), spectrographic and X-ray spectrographic, and radio-activation principles.

This order of presentation, together with a detailed table of contents and alphabetical list of 476 references, makes the subject matter readily accessible, even though the index is comparatively uninformative. A large amount of information has been compressed into a relatively small number of pages; under these circumstances, some detail must be sacrificed, but a slight amplification of statements, such as: "The method was developed for the analysis of a number of important materials," would have contributed even more to the book's usefulness.

It is not unexpected that a few printing errors are present in a first edition; much more disconcerting, however, is the occasional lapse from fluency in the translation. This is particularly evident in the chapters on polarography and spectroscopy, where the effect of too literal a translation has resulted in the use of words not readily accepted in the sense intended. Thus "medium" has become "average" in qualifying a spectrograph, with "tension" for "potential" and "clearing agent" where filter is intended.

These errors are unlikely to be misinterpreted by the experienced analytical or research chemist in industry or university, to whom the book will be of the greatest value, and only detract to a small extent from the potential usefulness of a monograph that contains a wealth of valuable information supported by the author's personal views and practical experience.

I. R. SCHOLES

Publications Received

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Based on a Symposium organised by The Metropolitan Microchemical Society under the sponsorship of The International Union of Pure and Applied Chemistry, Commission on Microchemical Techniques, Section of Analytical Chemistry, and supported in part by The National Institute of Health of the U.S. Public Health Service and held at The Pennsylvania State University, August 13th 18th, 1961.

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ORGANIC FUNCTIONAL GROUP ANALYSIS. By F. E. CRITCHFIELD. Pp. viii + 187. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 42s.

Report of the Analytical Methods Committee: Reprints

The Determination of Small Amounts of Copper in Organic Matter

THE Report prepared by the Metallic Impurities in Organic Matter Sub-Committee, "The Determination of Small Amounts of Copper in Organic Matter," reprinted from *The Analyst*, April, 1963, **88**, 253–258, is now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1. Price to members, 1s. 6d. each; to non-members, 2s. 6d. each.

Reports of the Analytical Methods Committee are only available from the Secretary (not through Trade Agents) and remittances, made out to the Society for Analytical Chemistry, MUST accompany orders.

Errata

APRIL (1963) ISSUE, p. 273, Fig. 3(*a*). The connection to the cathode of the EN 91 valve should be shown joined to the right-hand heater connection by the insertion of a dot at the point where the two connections cross.

MAY (1963) ISSUE, p. 397, 21st line from foot of page. *For* "sodium hydroxide" *read* "iodine".

AUGUST, 1963 Vol. 88, No. 1049

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

SECRETARYSHIP OF THE SOCIETY

THE Council has appointed Miss P. E. Hutchinson to be Secretary of the Society. Miss Hutchinson has been Assistant Secretary since 1955.

SECRETARIAT OF THE ANALYTICAL METHODS COMMITTEE

THE Council of the Society has appointed Mr. P. W. Shallis to be Secretary of the Analytical Methods Committee in place of Dr. C. H. Tinker (resigned). Mr. Shallis has been Assistant Editor of *The Analyst* since 1957.

NEW MEMBERS

ORDINARY MEMBERS

Roy Thomas Brittain, B.Pharm., M.P.S., M.I.Biol.; John Ivor Dennis; David John Dimmock; Geoffrey Edwin Downes, B.Sc.(Lond.), A.R.I.C.; John Esson, B.A., B.Sc.(Oxon.); Ralph Waldo France, B.Sc., Dip.Ed., Ph.D., F.Inst.P.; Derek Alfred Greenwood; George Rumbold Jamieson, B.Sc.(Lond.), F.R.I.C.; Geoffrey David Ratcliffe Jarrett; Allan Raymond Lister; Enzo Mannucci, Doc.Pharm.(Siena); John Michael Murphy, B.Sc.(Manc.); David John Pannett, B.Sc.(Sheff.); John Anthony Potter, A.R.I.C., Dip.App.Chem.; Lina Raffa; Ivor Smith, B.Sc., Ph.D.(Lond.), F.R.I.C., M.I.Biol.

JUNIOR MEMBER

Brian Thomas Ashurst, A.C.T.(Liv.), A.R.I.C.

DEATH

WE record with regret the death of

William Branch Pollard.

NORTH OF ENGLAND SECTION

THE twenty-sixth Summer Meeting of the Section was held at the Savoy Hotel, Blackpool, from Friday, June 14th, to Monday, June 17th, 1963.

The Chairman of the Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C., presided over an Ordinary Meeting at 10.30 a.m. on Saturday, June 15th, at which H. Pritchard, M.Sc., F.R.I.C., gave a lecture entitled "The Private Analyst and Public."

On the Saturday evening the party saw the Morecombe and Wise Show, and on the Sunday afternoon made a coach tour to Windermere, taking tea en route.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held on Thursday, June 13th, 1963, and took the form of a visit to the Control Laboratories of the United Dairies Ltd., Wood Lane, London, W.12.

The morning session was conducted under the Chairmanship of the Vice-Chairman of the Biological Methods Group, Dr. M. W. Parkes, B.Sc. After the Group had been welcomed by R. J. MacWalter, B.Sc., Ph.D.(Lond.), A.M.I.Chem.E., F.R.I.C., the following papers were read: "Aspects of the Natural Composition and Hygienic Quality Schemes," by R. C. Wright, B.Sc., Ph.D.; "Antibiotics in Milk," by J. Tramer, B.Sc., Ph.D.

After lunch the Group was conducted around the laboratories.

The thanks of the Group were expressed to the Company by Mr. W. A. Broom, B.Sc., F.R.I.C., Chairman of the Biological Methods Group.

Methods of Separation of Long-chain Unsaturated Fatty Acids

A Review*

By A. T. JAMES
(*Unilever Research Laboratory, Colworth House, Sharnbrook, Beds.*)

The isolation of virtually any unsaturated fatty acid can be accomplished on the gram scale by counter-current distribution involving use of the silver-ion containing solvent systems described by Dutton, Scholfield and Jones. The technique is, however, too slow for analysis and too cumbersome for amounts less than a gram. In this range, chromatographic procedures, especially liquid - liquid columns, thin-layer plates with the silver-ion complexing systems and gas - liquid chromatography are the methods of choice. Accurate and rapid analyses are best obtained by gas - liquid chromatography, but the combination of this technique with that of thin-layer chromatography is probably superior to all others.

MODERN analytical techniques have shown how complex are the mixtures of naturally occurring fatty acids. Indeed, the list is now so extensive that it almost rivals that of the petroleum hydrocarbons. The complexity of these mixtures places great demands on analytical and separation techniques.

The unique property of chromatographic systems lies in their ability to exploit relatively small differences in chemical structure between solute molecules. Unlike the older techniques of fractional distillation, crystallisation, etc., the chromatogram cannot conveniently deal with large quantities, but it can provide a quantitative yield of pure substances and is a better analytical tool. For these reasons this Review will be limited to counter-current distribution and the various chromatographic techniques. It is hoped to show the limitations of each of the individual techniques and suggest how they may be used to best advantage.

COUNTER-CURRENT DISTRIBUTION

This technique, developed originally by Craig, is capable of achieving purifications similar to those obtainable by chromatography. Its major disadvantages are cumbersomeness and slowness of operation. Its advantage lies in the large volumes of solvent that can be easily handled so that amounts of a few grams of a pure substance may be obtained. The plate efficiency of the counter-current machine is a direct function of the number of transfers employed, and the time of passage through a single plate is much slower than in a chromatographic column.

In an early paper Ahrens and Craig[1] described the separation of oleic, linoleic and linolenic acids with a solvent system of heptane, methanol, formamide and acetic acid. In this system, the distribution coefficient is altered to the same extent by the introduction of a double bond as by the removal of two CH_2 groups from the chain. Consequently, oleic and palmitic acids, and linoleic and myristic acids cannot be separated. A new type of solvent system has recently been introduced by Dutton, Scholfield and Jones,[2] who incorporate silver salts into the more aqueous of the two phases. A reversible complexing between the double bonds of unsaturated fatty acids and silver ions occurs, producing a large change in the distribution coefficient of the unsaturated fatty acids. This complexing occurs more readily with *cis* than with *trans* double bonds and hence affords a means of separating such isomers (see Figs. 1, 2 and 3). Many such procedures could be used to exploit more fully the capabilities of counter-current distribution.

CHROMATOGRAPHIC SYSTEMS

LIQUID - LIQUID COLUMNS—

The non-polar nature of the backbone of the long-chain fatty acids makes it necessary that the phases used possess a small dielectric constant. Suitable columns can be made only by having a relatively non-polar stationary phase. This approach was first exploited

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* Reprints of this paper will be available shortly. For details, please see p. 658.

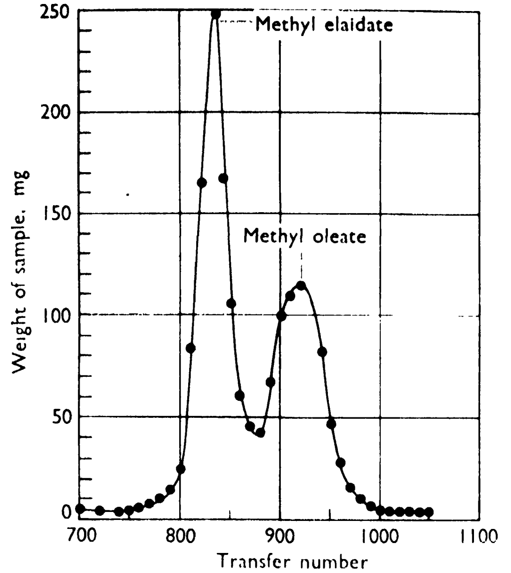

Fig. 1. Counter-current distribution in a 200-tube automatic instrument of methyl elaidate and methyl oleate between light petroleum and 0·2 M silver nitrate in 90 per cent. methanol. Sample was re-cycled to 700 transfers, and fractions were moved by the single withdrawal procedure (after Dutton, Scholfield and Jones[2])

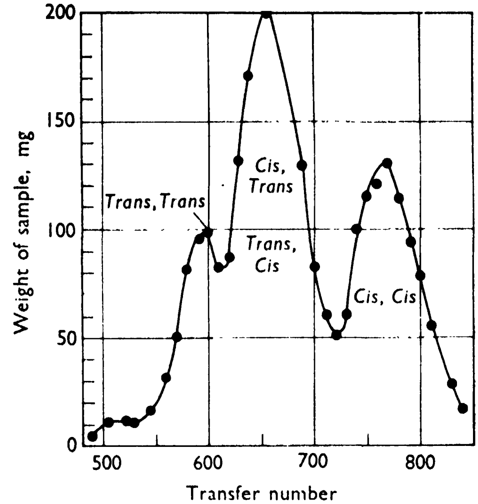

Fig. 2. Counter-current distribution of selenium-isomerised methyl linoleate between light petroleum and 0·2 M silver nitrate in 90 per cent. methanol. Sample was re-cycled to 450 transfers, and fractions were removed by the single withdrawal procedure (after Dutton, Scholfield and Jones[2])

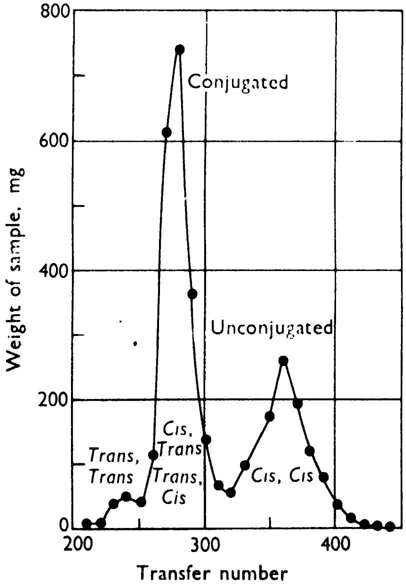

Fig. 3. Counter-currentid stribution of alkali-isomerised methyl linoleate between light petroleum and 0·2 M silver nitrate in 90 per cent. methanol. Fractions removed by the single withdrawal procedure (after Dutton, Scholfield and Jones[2])

by Howard and Martin,[3] who used liquid paraffin and aqueous acetone or methanol as the phase pairs, giving rise to the term "reversed phase" column, since all the earlier liquid - liquid chromatograms had the most polar liquid as the stationary phase. High molecular weight substances swellable by aqueous organic solvents can act not only as stationary hydrocarbon-like liquids, but also as the mechanical support, thus obviating the need for keiselguhr. Boldingh[4] used powdered rubber and Hirsch[5] used a powdered solid polymerised vegetable oil (Factice). In essence all these columns function in the same way and suffer from the disadvantage of the same overlap fractions described under counter-current distribution.

The silver-ion solvent systems described by Dutton *et al.*[2] would, when suitably modified, also give excellent separation of the unsaturated acids.

Paper chromatograms prepared with the same types of solvent system are subject to the same disadvantages; for a recent review see Viswanathan and Meera Bai.[6] Schlenk *et al.*[7] showed that chromatograms made at low temperatures gave improved separation of the saturated and unsaturated fatty acids owing to a greater change in distribution coefficient of the unsaturated acids.

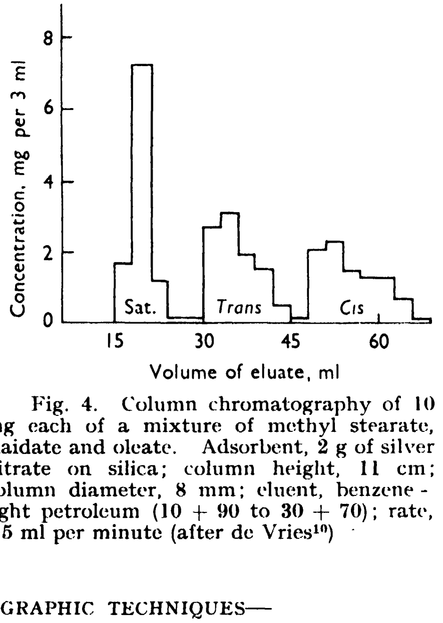

Fig. 4. Column chromatography of 10 mg each of a mixture of methyl stearate, elaidate and oleate. Adsorbent, 2 g of silver nitrate on silica; column height, 11 cm; column diameter, 8 mm; eluent, benzene - light petroleum (10 + 90 to 30 + 70); rate, 0·5 ml per minute (after de Vries[10])

LIQUID - LIQUID CHROMATOGRAPHIC TECHNIQUES—

Thin-layer chromatography and silica gel columns give similar types of separation, the former giving higher resolution. Such columns are capable of resolving cholesterol esters of saturated and unsaturated acids, *e.g.*, saturated acids are eluted as a group followed by oleate, linoleate and arachidonate.[8] Simple esters could be similarly resolved.

Thin-layer chromatography of fatty acid methyl esters after formation of the acetoxymercuri-methoxy derivatives (produced by reaction between the unsaturated acids and mercuric acetate) gives a group separation of saturated and unsaturated acids with a light petroleum - diethyl ether (4 + 1 v/v) moving phase.[9] After the plate has been dried a second solvent flowing in the same direction (n-propanol - glacial acetic acid, 100 + 1 v/v) and gives a separation of monoenoic acids (R_F 0·85), dienoic acids (a double spot, R_F 0·45 and 0·55) and trienoic acids (R_F 0·15), whereas more highly unsaturated acids remain at the origin. Larger amounts can be obtained by streaking a sample across the plate.

The use of silver ion complexes of the unsaturated acids has greatly improved separations. de Vries[10] described good separations of methyl esters of unsaturated acids and glycerides on silver nitrate impregnated silicic acid columns (see Figs. 4 and 5). Such complexes have also been used by Morris[11] on thin-layer plates, showing excellent separation of *cis-trans* isomers and of unsaturated acids of increasing degree of unsaturation (see Figs. 6 and 7).

Such thin-layer and column techniques in conjunction with gas - liquid chromatography represent the most powerful combination suitable for the isolation of milligram amounts of unsaturated fatty acids.

GAS-CHROMATOGRAPHIC SEPARATIONS—

One of the major advantages of the gas - liquid chromatogram is that the moving phase is a permanent gas whose interactions with the solutes can be ignored. The solubility of the moving phase in the stationary phase is so small that this can also be ignored, and the only interaction forces that need be considered are interactions between solute and solvent. It is for this reason that the relation between chromatographic behaviour and structure of solute and solvent are more readily understandable in gas - liquid than in other types of chromatography.

SOLUTION EFFECTS IN THE LIQUID PHASE—

There are two major classes of solute - solvent interactions that need be considered. First, London Dispersion Forces, which occur in all solvents. These interactions are due primarily to the vibration of bonds setting up oscillating dipoles and so giving rise to weak interaction forces at low intermolecular distances. For a given molecular weight solvent the forces increase with increasing molecular weight of solute and decrease with chain branching. With cyclic structures large entropy effects become manifest, such that the apparent interaction energy is greater for a cyclic structure than for its straight-chain isomer. In this simplified treatment, which is confined to observations on the relative rates of movement of saturated and unsaturated fatty acids, such entropy effects will be ignored. The total London Dispersion Interaction of a long-chain saturated acid in a non-polar solvent, such as the Apiezon greases, is much greater than the total of the polar interaction forces considered in the next section.

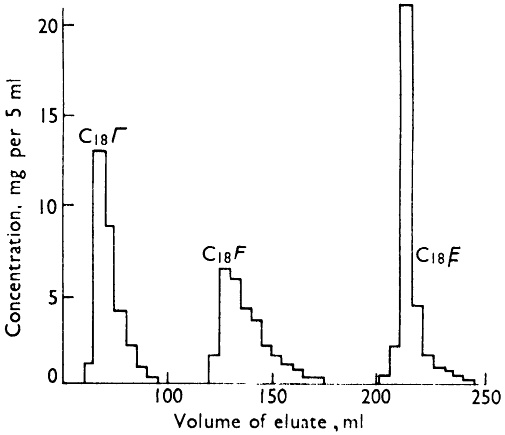

Fig. 5. Column chromatography of 30 mg each of a mixture of methyl oleate, linoleate and linolenate. Adsorbent, 10 g of silver nitrate on silica; column height, 23 cm; column diameter, 14 mm; eluent, benzene - light petroleum (40 + 60 to 100 + 0); rate, 0·5 ml per minute (after de Vries[10])

Secondly, there are polar interaction forces. It is unusual in gas chromatography to deal with formally charged solutes, since these have too small a partial pressure. It is also unusual to deal with molecules possessing large formal dipoles. The major class of interaction forces between polar or polarisable solutes and solvents are of the electron donor - acceptor type. In these interactions, as would be expected from the name, one molecule partially donates and shares a bonding electron with an acceptor molecule, thus setting up a very weak reversible bond. The electron transitions involved in such complexes would be expected to bear some relation to the transition state in chemical reactions. However, no bond re-arrangement occurs, but the known effects of substituent groups in controlling chemical reactivity would be expected to facilitate or obstruct the formation of such complexes. The hydrogen bond is one of the best known of such donor - acceptor bonds. The double bonds of unsaturated fatty acids show electron donor - acceptor interactions with the ester groups of the polyester stationary phases. The interaction is specific but not very large. The total interaction energy of a fatty acid methyl ester with a polyester is less than with a hydrocarbon stationary phase (see Table I). (The interaction energy is a function of the corrected retention volume.)

In the next section a simplified theoretical treatment of the interaction of solute and solvent has been attempted.

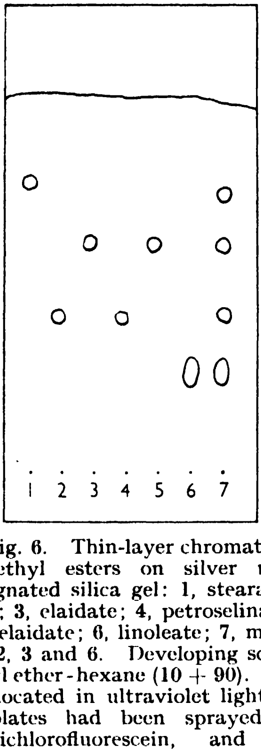

Fig. 6. Thin-layer chromatogram of methyl esters on silver nitrate impregnated silica gel: 1, stearate; 2, oleate; 3, elaidate; 4, petroselinate; 5, petroselaidate; 6, linoleate; 7, mixture of 1, 2, 3 and 6. Developing solvent, diethyl ether-hexane (10 + 90). Spots were located in ultraviolet light after the plates had been sprayed with 2′,7′-dichlorofluorescein, and were reproduced by tracing (after Morris[11])

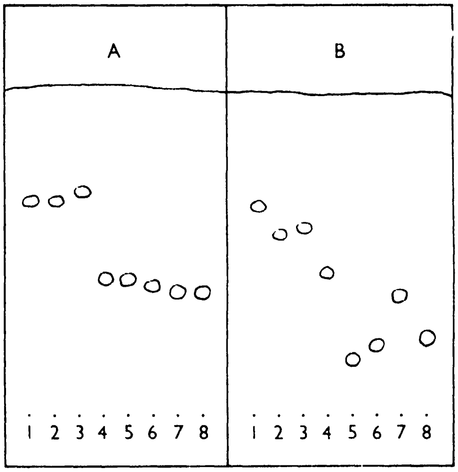

Fig. 7. Thin-layer chromatogram of methyl esters on silica gel (A) and silver nitrate impregnated silica gel (B): 1, *cis*-9,10-epoxyoctadecanoate; 2, *cis*-9,10-epoxyoctadec-12-enoate; 3, *cis*-12,13-epoxyoctadec-9-enoate; 4, 12-hydroxyoctadecanoate; 5, 12-hydroxyoctadec-9-enoate; 6, 12-hydroxyoctadec-9-enoate; 7, 9-hydroxyoctadecanoate; 8, 9-hydroxyoctadec-12-enoate. Developing solvent, diethyl ether - hexane (40 + 60). Spots were located in ultraviolet light after the plates had been sprayed with 2′,7′-dichlorofluorescein, and were reproduced by tracing (after Morris[11])

TABLE I

SPECIFIC RETENTION VOLUMES OF METHYL MYRISTATE PER GRAM OF STATIONARY PHASE AT COLUMN TEMPERATURE

Stationary phase	V_g, ml
Apiezon L at 197° C	1320
Polyethylene glycol adipate at 180° C	507

SEPARATION FACTORS AND CHEMICAL STRUCTURE—

The rate of movement of any substance along a column is governed by its distribution coefficient. The latter is controlled by the free energy required to transfer a molecule of solute from solution in the column stationary phase to the gas phase.

Provided the concentration is small enough for the distribution coefficient to be independent of concentration (*i.e.*, the necessary condition for symmetrical peaks) then Raoult's law can be assumed.

Then—

$$\mu_A^S = \mu_A^{S0} + RT\,\mathrm{Ln}\,N_A^S \text{ (derived by Martin}^{12}\text{)},$$

where μ_A^S is the chemical potential of substance A in either phase, μ_A^{S0} is its chemical potential in the same standard state and N_A^S is the mole fraction of A in the phase in question. If the gas phase and liquid phase are in equilibrium then—

$$\mu_A^G - \mu_A^L = 0,$$

$$\text{which} = \mu_A^{G0} - \mu_A^{L0} + RT\,\mathrm{Ln}\,N_A^G - RT\,\mathrm{Ln}\,N_A^L\,.$$

If $\mu_A^{G0} - \mu_A^{L0} = \Delta\mu_A$.

Then $\Delta\mu_A = RT\,\mathrm{Ln}\,\dfrac{N_A^L}{N_A^G}$.

But $\dfrac{N_A^L}{N_A^G} = K$, the partition coefficient.

Then $\mathrm{Ln}\,K_A = \dfrac{\Delta\mu_A}{RT}$, where $\Delta\mu_A$ is the free energy required to transport 1 mole of A from the liquid phase to the gas phase.

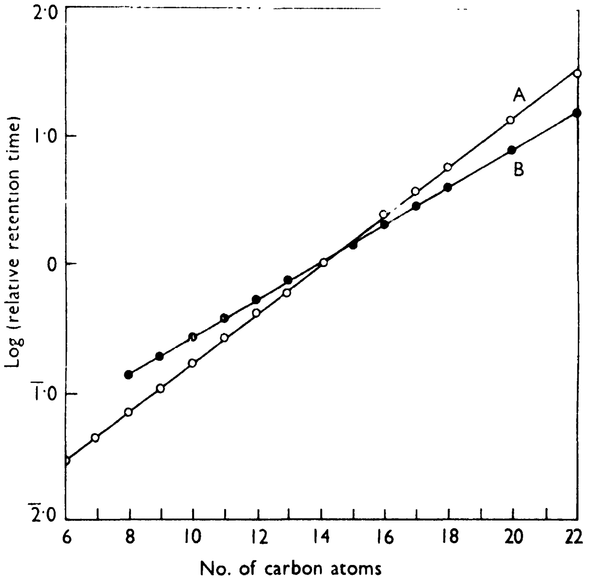

Fig. 8. Relation between chain length and the logarithm of the relative retention time on saturated fatty acids in: A, a non-polar medium (Apiezon L); B, a polar medium (polyethylene glycol adipate)

If a molecule, B, differs from A by possession of a substituent group, *X*, then—

$$\mathrm{Ln}\,K_B = \frac{\Delta\mu_B}{RT}$$

$$\text{and } \mathrm{Ln}\,K_B - \mathrm{Ln}\,K_A = \frac{1}{RT}\,(\Delta\mu_B - \Delta\mu_A)$$

$$= \frac{1}{RT}\,(\Delta\mu_X)$$

where $\Delta\mu_X$ is the free energy necessary to move the group *X* from one phase to the other.

Now $K_A = C_1\,(V_R^0)_A$ where C_1 is the column constant and V_R^0 is the corrected retention volume (volume of gas required to elute substance A from the column).

Then—

$$\mathrm{Ln}\,\frac{K_B}{K_A} = \mathrm{Ln}\,\frac{C_1\,(V^0_R)_B}{C_1\,(V^0_R)_A}$$

= Ln retention volume of substance B relative to substance A. This is dependent only on the temperature and the chemical nature of the stationary phase.

Thus $\log_{10}$ (retention volume) of B relative to A

$$= \frac{\Delta\mu_x}{2{\cdot}3\,RT}.$$

If the group X can be added to molecule A without causing any perturbation of electronic configuration in atoms other than that to which X is attached, then addition of a further X group should give the same increase in relative retention volume.

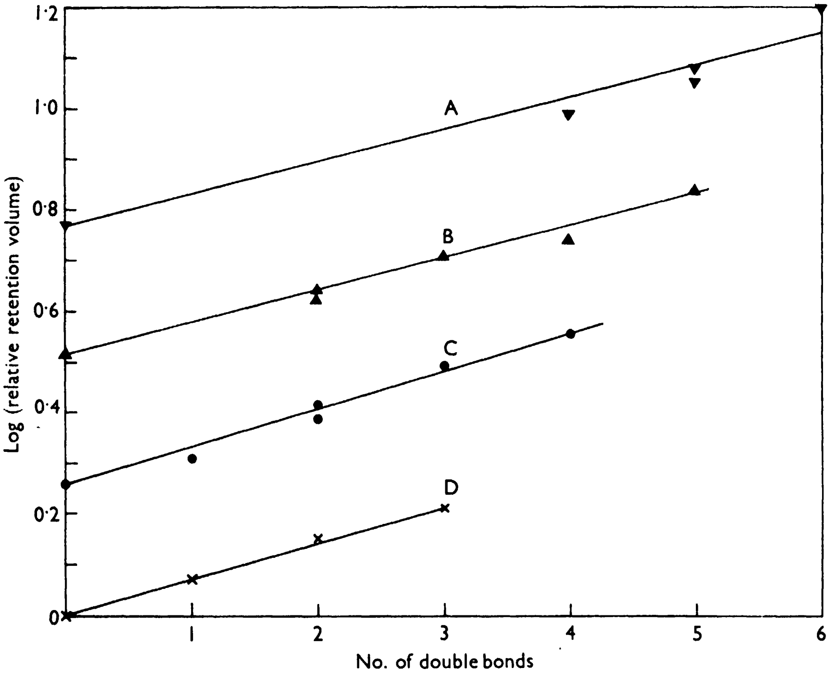

Fig. 9. Relation between the logarithm of the relative retention volume and the number of double bonds in fatty acids having: A, 22 carbon atoms; B, 20 carbon atoms; C, 18 carbon atoms; D, 16 carbon atoms

EFFECT OF INCREASE IN CHAIN LENGTH—

Increasing the length of a chain by a $-CH_2-$ group would thus be expected to give the same relative increase in retention volume irrespective of chain length. This is demonstrated in Fig. 8, where chain length is plotted against $\log_{10}$ (relative retention volume) for long-chain saturated acid methyl esters in two stationary phases. The largest $-CH_2-$ separation factor is given by the non-polar stationary phase (Apiezon L grease) in which the London Dispersion Interaction is at its maximum. The $-CH_2-$ separation factor is purely a London Dispersion effect, since the group is unable to show any polar interaction. In the polyester (ethylene-glycol adipate) stationary phase the $-CH_2-$ separation factor is lower since the total London Dispersion Interaction in this phase is less than in the non-polar phase.

EFFECT OF INSERTION OF DOUBLE BONDS—

In the hydrocarbon non-polar stationary phases, introduction of a double bond decreases the retention volume compared with the corresponding saturated fatty acid. This would be expected since the maximum interaction will occur between $-CH_2-$ groups of solute and solvent, and $-CH = CH-$ groups will have a smaller interaction.

In a stationary phase such as a polyester, which shows an interaction between its ester groups and the double bonds of the solute, introduction of the double bond into the solute molecule causes an increase of retention volume. This effect is opposed by the London Dispersion Interaction, which is working in the opposite direction. A plot of log (retention volume) against the number of double bonds in molecules of different chain length is shown in Fig. 9, demonstrating that introduction of each double bond gives approximately the same increase in relative retention volume. This is true only for double bonds separated by a CH_2 group and not for conjugated systems. The separation factors for introduction of both CH_2 groups and double bonds are shown in Table II.

TABLE II

INCREASES IN RELATIVE RETENTION VOLUME BROUGHT ABOUT BY INTRODUCTION OF $-CH_2-$ AND $-CH{=}CH-$ GROUPS

Increases expressed as retention volume relative to the parent saturated acid

Group	Apiezon L at 197° C	Apiezon M at 197° C	Polyethylene glycol succinate at 180° C	Polyethylene glycol adipate at 180° C	Reoplex 400 at 197° C
$-CH_2-$	2·36	2·39	1·66	1·97	1·8
$-CH{=}CH-$	0·9	0·89	1·17	1·18	1·13

EFFECT OF CHANGE OF DOUBLE BOND POSITION—

In non-polar stationary phases there is a small but detectable difference in retention volume between the isomeric monoenoic acids (see Table III). Thus the Δ^4- and Δ^6-octadecenoic acids have slightly lower retention volumes than has the Δ^9- acid. The effect with polyester stationary phases is much smaller. With dienoic acids, both types of stationary phase are capable of differentiating between positional isomers (see Table IV). With trienoic acids on the other hand, change of position of the double bonds does not appear to alter the retention volume (see Table V). With tetraenoic acids there is again a small positional effect (see Table VI).

TABLE III

EFFECT ON RETENTION VOLUME OF DOUBLE BOND POSITION IN MONOENOIC ACIDS

Retention volumes relative to methyl palmitate

Fatty acid	Apiezon L at 197° C	Apiezon M at 197° C	PEGA at 180° C	PDEGS at 200° C	Reoplex 400 at 197° C
$16:1^7$ *cis*		—	1·15	1·18	1·13
$16:1^8$ *cis*	—	0·89	—	—	—
$16:1^9$ *cis*	0·90	0·89	1·15	1·18	1·13
$18:1^9$ *cis*	2·03	2·08	2·21	2·00	—
$18:1^6$ *cis*	—	2·05	2·22		—
$18:1^4$ *cis*		2·05	—	—	—

TABLE IV

EFFECT ON RETENTION VOLUME OF DOUBLE BOND POSITION IN DIENOIC FATTY ACIDS

Acid	Apiezon M at 197° C	PEGA at 184·5° C	Reoplex 400 at 197° C
$16:2^{6,9}$	0·86	1·45	1·28
$16:2^{9,12}$	0·89	1·45	1·39
$18:2^{6,9}$	1·81	2·76	2·56
$18:2^{9,12}$	1·88	2·60	2·32
$20:2^{8,11}$	4·17	4·22 (197° C)	—
$20:2^{11,14}$	4·38	4·46 (197° C)	—

BOND CONJUGATION—

Relatively little work has been carried out with conjugated fatty acids of known structure. Beerthuis *et al.*[13] have studied the behaviour of conjugated C_{18} dienoic and trienoic acids on an Apiezon L column. The relative retention volumes they obtained are shown in Table VII and suggest that double bond position has less effect than conjugation and that *cis-trans* structures overlap *trans-cis* structures. The trienoic acids are well separated from the corresponding dienoic acids, unlike the unconjugated acids.

TABLE V

EFFECT ON RETENTION VOLUME OF DOUBLE BOND POSITION IN TRIENOIC FATTY ACIDS

Acid	Apiezon M at 197° C	PEGA at 184·5° C
$16:3^{6,9,12}$	0·77	1·72
$16:3^{7,10,13}$	0·77	1·72
$18:3^{6,9,12}$	1·88	—
$18:3^{9,12,15}$	1·88	—
$20:3^{5,8,11}$	3·89	-
$20:3^{8,11,14}$	3·89	—

TABLE VI

EFFECT ON RETENTION VOLUME OF DOUBLE BOND POSITION IN TETRAENOIC AND PENTAENOIC ACIDS

Acid	Apiezon M at 197° C	Reoplex 400
$20:4^{5,8,11,14}$	3·48	5·28
$20:4^{8,11,14,17}$	3·48	5·35
$22:5^{7,10,13,16,19}$	7·82	11·7
$22:5^{5,8,11,14,17}$	7·88	12·2

EFFECT OF *cis-trans* ISOMERISM ON RETENTION VOLUME—

In non-polar stationary phases the *trans* acids, since they possess a more extended configuration than the corresponding *cis* acids, show a greater interaction and have a retention volume greater by a factor of approximately 1·05 in monoenes, 1·2 in conjugated dienes and 1·17 in conjugated trienes (see Table VIII). In the polyester stationary phases the major interaction is between the double bond and the ester groups. A change of configuration of the hydrocarbon chains about the double bond does not alter the retention volume of the monoenes.

Litchfield, Isbell and Reiser[14] have recently published a study of the gas chromatographic behaviour of geometrical isomers of $\Delta^{9,12}$-octadecadienoic acid. Their results indicate the expected retardation of the *cis-trans* compound relative to the *cis-cis* acid, but an unexpected overlap of the *trans-cis* and *trans-trans* acids on an Apiezon L capillary column. On a diethylene glycol succinate polyester column the *cis-cis* and *cis-trans* acids overlap, but the *trans-cis* and *trans-trans* acids are separable. By using both columns all the isomers can be resolved, the results expressed as carbon numbers[15] are shown in Table IX.

TABLE VII

RETENTION VOLUMES OF CONJUGATED ACIDS ON APIEZON L AT 197° C

Acid	Retention volume relative to methyl palmitate
$18:2^{9,12}$ *cis-cis*	1·90
$18:0$	2·36
$18:2^{10,12}$ *trans-cis*, $18:2^{9,11}$ *cis-trans*	2·56
$18:2^{10,12}$ *trans-trans*, $18:2^{9,11}$ *trans-trans*	3·04
$18:3^{9,11,13}$ *cis-trans-trans*	3·82
$18:3^{9,11,13}$ *trans-trans-trans*	4·43

OVERLAPPING OF SATURATED AND UNSATURATED FATTY ACIDS—

It would be too much to hope for a separation of all the possible saturated and unsaturated acids in one pass through a gas - liquid column. Both polyester and Apiezon columns show some overlaps. These are listed in Table X.

STABILITY OF UNSATURATED FATTY ACIDS ON GAS - LIQUID CHROMATOGRAMS—

Some comparisons have been made between the results obtained from Apiezon L columns and the alkali isomerisation techniques. No indications have been found of any breakdown or loss of acids containing up to 4 double bonds on these columns. Condensation of the peaks emerging and subsequent infrared and ultraviolet examination has not disclosed any significant bond re-arrangement. The same is true for polyester stationary phases, but here it is necessary to work at as low a temperature as is possible consonant with the required speed of analysis, since it has been shown that there is a progressive loss of slower moving acids when column temperatures exceed 200° C.

TABLE VIII

EFFECT OF GEOMETRICAL ISOMERISM OF THE DOUBLE BONDS ON RELATIVE RETENTION VOLUME (METHYL PALMITATE = 1)

Acid	Apiezon L at 197° C	Apiezon M of 197° C	PEGA at 180° C	Apiezon L at 150° C	DEGS at 120° C
$16:1^9$ *cis*	0·90	—	1·15	—	—
$16:1^9$ *trans* ..	0·92	—	1·15	—	—
$18:1^9$ *cis*	2·03	—	2·21	—	—
$18:1^9$ *trans* ..	2·12	—	2·21	—	—
$18:1^4$ *cis*	—	2·05	—	—	—
$18:1^4$ *trans* ..	—	2·14	—	—	—
$10:1^2$ *cis*	—	—	—	1·00*	1·06*
$10:1^2$ *trans* ..	—	—	—	1·37*	1·83*
$10:1^3$ *cis*		—	—	0·93*	1·38*

* Figures published by Lennarz *et al.*[16] relative to methyl decanoate.

TABLE IX

CARBON NUMBERS OF THE GEOMETRICAL ISOMERS OF METHYL LINOLEATE (AFTER LITCHFIELD *et al.*[14])

Compound	Apiezon L at 200° C	DEGS at 175° C
^{9,12} *cis-cis*-octadecadienoate	17·48	19·46
^{9,12} *cis-trans*-octadecadienoate	17·59	19·46
^{9,12} *trans-cis*-octadecadienoate	17·64	19·55
^{9,12} *trans-trans*-octadecadienoate	17·64	19·35

TABLE X

SOME UNRESOLVED MIXTURES

Acid	Apiezon	Polyesters
Iso-branched saturated of *x* carbon atoms .. .. .. Monoenoic acids of *x* carbon atoms	Overlap	Resolved
Dienoic and trienoic, *x* carbon atoms	Overlap	Resolved
Tetra- and pentaenoic, *x* carbon atoms	Overlap	Resolved
Cis and *trans* isomers of monoenoic acids	Resolved	Overlap
Trienoic acids of *x* carbon atoms and saturated acids of *x* + 2 carbon atoms	Resolved	Partially resolved

CHOICE OF STATIONARY PHASE—

It may be seen from the above that neither the polyester nor the hydrocarbon phases are capable of separating all the possible saturated and unsaturated fatty acids. Whenever possible, both types of column should be used. If this cannot be done, then the type of column capable of giving the maximum information for the particular fatty acid mixture should be used. For example, when *cis-trans* isomers are likely to exist, the Apiezon column would be best; when separations of mono-, di-, tri- and more highly unsaturated acids are required, then the polyester phases would be best. A wide variety of different polyesters have been used, but the major ones in use now are—

Polyethylene glycol adipate (PEGA).
Polyethylene glycol succinate (PEGS).
Polydiethylene glycol succinate (polybutanediol succinate) (PDEGS).

When there is overlap on one column the components can almost always be resolved on the other type of column. Thus linolenic and arachidic acids incompletely separated on most polyester columns can be cleanly resolved on an Apiezon column. The same is true in reverse, the di- and trienoic, and tetra- and pentaenoic acids overlap on Apiezon columns. When doubt exists as to the structure of an acid from its chromatographic position alone, then measurement of its relative retention volume on the two types of column will give information as to both the number of carbon atoms and the number of double bonds in the molecule.[17] Positional isomers of unsaturated acids have very similar relative retention volumes, so that chromatographic information alone is not sufficient conclusively to define structure. This is best done by collection of the substances from a gas chromatogram, with subsequent oxidative degradation and identification of the fragments by gas chromatography.[18,19,20]

CAPILLARY COLUMNS—

Capillary columns, when operated in such a manner as to produce very high plate numbers, can sometimes help in identification, because of the better separation obtained with small separation factors. Since, however, the small internal diameter columns can carry only small charges, they are not so useful when isolated fractions are required. Lipsky, Lovelock and Landowne[21] have described high efficiency Apiezon L capillary columns showing an almost complete separation of methyl oleate and methyl elaidate.

I thank Dr. H. J. Dutton, Dr. B. de Vries, Dr. L. J. Morris and the Editors and Publishers of *Chemistry and Industry* for permission to reproduce diagrams.

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Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factors for Chicken

THE Analytical Methods Committee has received the following report from its Meat Products Sub-Committee. The report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Mr. H. C. Hornsey, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams, with Dr. C. H. Tinker as Secretary.

As in its reports on pork[1] and beef,[2] the Sub-Committee agreed to base on Stubbs and More's method its recommendations for the determination of the raw fresh meat content in products made from chicken meat. A nitrogen factor of 3·5 is generally used for such determinations, and the Sub-Committee decided to test the validity of this factor and, if necessary, to recommend the use of alternative factors.

Again, the Sub-Committee was fortunate in obtaining the collaboration of several laboratories of meat-product manufacturers (see below), who agreed to analyse chicken carcases according to the requirements of the Sub-Committee.

The Sub-Committee recommended that older heavier birds should be examined, as it is unusual for young light birds to be used for manufacturing purposes. It was also arranged that the breast meat should be analysed separately from the other meat, as breast meat and dark meat are sometimes used separately for manufacturing purposes.

The instructions listed below were issued to the collaborating laboratories.

PROCEDURE FOR THE PREPARATION OF SAMPLES

1. The neck, head and feet should be removed from the already eviscerated and plucked chicken.

2. The breast meat should be dissected completely, weighed, and then finely minced and mixed.

3. All other flesh and skin (exclusive only of any adhering intestinal fat), should then be completely dissected from the bones, weighed, finely minced and then mixed.

4. Portions from 2 and 3 should then be weighed out for moisture, fat, ash and nitrogen determinations.

5. All these operations should be performed with a minimum of delay, to minimise evaporation losses.

6. When possible, the following details should be supplied—

(*a*) Weight and age of bird.

(*b*) Weights of "breast meat" and "other meat."

(*c*) Percentages of moisture, fat, ash, nitrogen and nitrogen expressed on the fat-free, on both the "breast meat" and the "other meat."

RESULTS

The results collected by the Sub-Committee are shown in Fig. 1. Those marked with an asterisk were obtained on samples taken by the procedure outlined above, and the values thus marked in the "Whole Carcase" section of the chart have been calculated from the values for the component parts. In addition, the values marked with a dagger in this section were obtained on composite samples prepared by dissecting and comminuting all flesh and skin from the entire carcase. In other tests sampling was not carried out by the prescribed method, since the analyses were completed before the instructions had been issued. The resulting figures may be to some extent affected by this variation; it would, for instance, be reasonable to assume that the average figure for leg meat found by Laboratory C would have been higher had the skin not been removed.

The Sub-Committee was of the opinion that it should allow for the different use of breast meat, and that it should recommend different nitrogen factors for breast meat and all the other meat. When no information is available on the kind of chicken meat used, a general factor would have to be applied.

RECOMMENDATION

The Sub-Committee recommends the following average figures for use in the analyses, of chicken products: breast meat, 3·9; dark meat, 3·6; whole carcase 3·7.

ACKNOWLEDGMENT

The Sub-Committee thanks those listed below for their help and communications—

Brand & Co. Ltd.
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J. Lyons & Co. Ltd.
The Official Norwegian Quality Control Institute for Canned Fish Products.
S. J. Palmer (N/C) Ltd.
J. Sainsbury Ltd.
C. Shippam Ltd.
Unilever Ltd.

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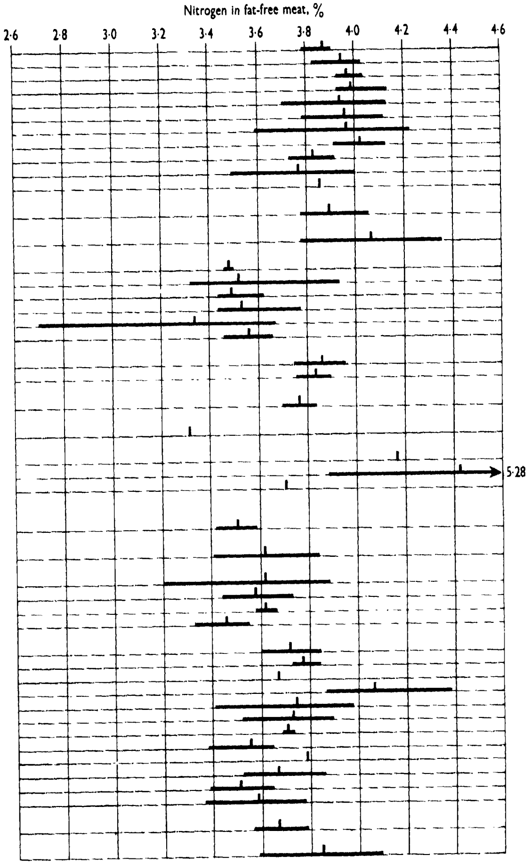
			No. of samples	Range	Mean	
BREAST MEAT	Excess of fat removed	Laboratory A	3	3·79 to 3·90	3·86	3·92
	Home killed	Laboratory A	4	3·82 to 4·02	3·94	
	From different regular sources. fat excluded as far as possible	Laboratory B	7	3·92 to 4·03	3·96	
		Laboratory B	8	3·92 to 4·13	3·98	
	Hens 31 to 55 months: skin and easily removable fat discarded	Laboratory C	13	3·70 to 4·13	3·93	
	*Hens: Light Sussex: 26 to 27 months	Laboratory C	12	3·78 to 4·11	3·95	
	*Hens: 1½ to 2 years: 5 to 6 lb	Laboratory D	6	3·59 to 4·22	3·96	
	Boiling fowl	Laboratory E	2	3·91 to 4·12	4·02	
	*Boiling fowl· Rhode Island Red: 3¼ to 4½ lb	Laboratory E	4	3·72 to 3·91	3·82	
	*Boiling fowl: Light Sussex: 2¾ to 5¼ lb	Laboratory E	6	3·48 to 4·00	3·76	
	Dutch hens: average weight 3¾ lb	Laboratory F	10	S.D. 0·074	3·85	
	Norwegian hens: White Italians: 2½ years: 2¼ to 4 lb: battery reared: skin discarded	Laboratory K	5	3·77 to 4·05	3·89	
	Norwegian hens· White Italians. 2½ to 4 years. 2¼ to 3½ lb: free range: skin discarded	Laboratory K	5	3·77 to 4·35	4·06	
LEG MEAT	Excess of fat removed	Laboratory A	2	3·46 to 3·50	3·48	3·45
	Home killed	Laboratory A	4	3·32 to 3·84	3·52	
	From different regular sources: fat excluded as far as possible	Laboratory B	7	3·44 to 3·61	3·49	
		Laboratory B	8	3·44 to 3·77	3·53	
	Hens· 31 to 55 months. skin and easily removable fat discarded	Laboratory C	13	2·70 to 3·67	3·34	
	Boiling fowl	Laboratory E	2	3·46 to 3·66	3·56	
WING MEAT	Home killed	Laboratory A	2	3·75 to 3·96	3·86	3·84
	Boiling fowl	Laboratory E	2	3·76 to 3·90	3·83	
BACK MEAT	Boiling fowl	Laboratory E	2	3·70 to 3·84	3·77	
NECK MEAT	Dutch hens average weight 3¾ lb	Laboratory F	1		3·32	
SKIN		Laboratory A	1		4·17	4·31
	Boiling fowl	Laboratory E	6	3·89 to 5·28	4·43	
	Dutch hens average weight 3¾ lb	Laboratory F	1		3·71	
ALL MEAT OTHER THAN BREAST	Norwegian hens· White Italians: 2¼ to 4 lb. battery reared: skin discarded	Laboratory K	5	3·42 to 3·59	3·51	3·57
	Norwegian hens· White Italians: 2½ to 4 years: 2¼ to 3½ lb: free range. skin discarded	Laboratory K	5	3·41 to 3·84	3·62	
ALL MEAT OTHER THAN BREAST (INCLUDING SKIN)	*Hens· Light Sussex 26 to 27 months	Laboratory C	12	3·20 to 3·89	3·62	3·58
	Hens 1½ to 2 years 5 6 lb	Laboratory D	6	3·45 to 3·73	3·58	
	*Boiling fowl: Rhode Island Red: 3¼ to 4½ lb	Laboratory E	4	3·58 to 3·67	3·62	
	*Boiling fowl Light Sussex: 2¾ to 5¼ lb	Laboratory E	6	3·33 to 3·55	3·46	
WHOLE CARCASE	Female· home killed	Laboratory A	7	3·60 to 3·85	3·72	3·71
	Excess of fat removed	Laboratory A	3	3·73 to 3·84	3·77	
	Hen. 5 lb· excess of fat removed· half of carcase analysed	Laboratory A	1		3·67	
	†Chickens· 9 to 12 months: skin included	Laboratory C	5	3·87 to 4·38	4·07	
	*Hens· Light Sussex: 26 to 27 months	Laboratory C	12	3·41 to 3·98	3·75	
	*Hens. 1½ to 2 years: 5 to 6 lb	Laboratory D	6	3·52 to 3·90	3·73	
	*Boiling fowl. Rhode Island Red: 3¼ to 4½ lb	Laboratory E	4	3·69 to 3·73	3·71	
	*Boiling fowl· Light Sussex: 2¾ to 5¼ lb	Laboratory E	6	3·38 to 3·65	3·56	
	"Sampled proportionately as far as possible"	Laboratory G	1		3·79	
		Laboratory H	3	3·53 to 3·87	3·67	
	†Broilers. approx. 2 lb: skin included	Laboratory J	6	3·39 to 3·65	3·52	
	†Chickens. approx 3¾ lb: skin included	Laboratory J	8	3·37 to 3·78	3·59	
	Norwegian hens: White Italians: 2½ years: 2¼ to 4 lb: battery reared. skin discarded	Laboratory K	5	3·57 to 3·79	3·67	
	Norwegian hens· White Italians: 2½ to 4 years: 2¼ to 3½ lb: free range: skin discarded	Laboratory K	5	3·59 to 4·10	3·85	



* Samples taken by proposed procedure

† Composite samples prepared by dissecting and comminuting all flesh and skin from entire carcase

Fig. 1. Nitrogen on fat-free contents of various portions of chicken. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values

n-Propyl Gallate as a Gravimetric Reagent for Bismuth and Antimony

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The use of n-propyl gallate as a reagent for separating and determining bismuth and antimony in the presence of interfering cations has been examined. Quantitative recovery of bismuth is achieved from 0·01 N nitric acid solution and of antimony from 0·5 N hydrochloric acid solution.

A proposed structure for these compounds is suggested, based on the results of infrared analysis. Applications of the propyl gallate gravimetric method to the analysis of alloys and pharmaceuticals are discussed.

POLYHYDRIC phenolic compounds have found wide application in analytical chemistry as gravimetric and colorimetric reagents. Pyrogallol and gallic acid have been proposed as precipitants for bismuth and antimony,[1,2,3,4] but it is well known that solutions of pyrogallol are unstable. Kieft and Chandlee[4] have reported that gallic acid decomposes in aqueous solution above 85° C. A polarographic study by Nash, Skauen and Purdy[5] of the anodic oxidation of phenolic compounds at a wax-impregnated graphite electrode showed that n-propyl gallate was more resistant to oxidation than was gallic acid, which in turn was more stable than pyrogallol. Our qualitative observations on aqueous solutions of these reagents are in accordance with this work and suggest that n-propyl gallate is to be preferred to gallic acid and pyrogallol as an analytical reagent.

n-Propyl gallate is available in the form of hydrated crystals, m.p. 65° C, or as an anhydrous powder, m.p. 147° C. It dissolves readily in warm water and gives, with various inorganic cations, precipitates whose solubilities are dependent on pH. Table I presents those reactions observed for the group-2 cations in nitric acid solutions and in buffered acetic acid at pH 5.

TABLE I

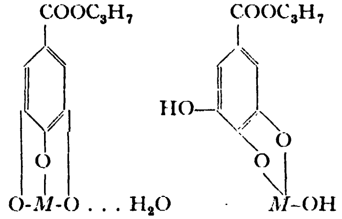
PRECIPITATION OF METALS BY n-PROPYL GALLATE

The concentration of the metals was 0·2 mg per ml

Metal	In N nitric acid	In 0·01 N nitric acid	In acetic acid - acetate buffer at pH 5·0
Zn^{II}*	No precipitate	No precipitate	No precipitate
Cd^{II}*	No precipitate	No precipitate	No precipitate
Pb^{II}	No precipitate	No precipitate	White precipitate
Cu^{II}	No precipitate	No precipitate	Brown precipitate
Hg^{I}	No precipitate	No precipitate	Yellow precipitate
Hg^{II}	No precipitate	Yellow precipitate	Yellow precipitate
Bi^{III}	No precipitate	Yellow precipitate	Yellow precipitate
Sb^{III}	White precipitate (forms slowly)	White precipitate	White precipitate
As^{III}	No precipitate	No precipitate	No precipitate
Sn^{II}	No precipitate	Opalescence	White gelatinous precipitate
Sn^{IV}	No precipitate	Opalescence	White gelatinous precipitate

* Forms precipitate with n-propyl gallate in neutral solution.

Bismuth and antimony have been found to yield flocculent precipitates that can readily be collected by filtration and can be dried in sintered-glass or porous-porcelain Gooch crucibles at 110° C to give stoicheiometric compounds of the generic formula $C_{10}H_{11}O_6.M$, which could obviously be represented by either the monohydrate structure (I) or the subgallate structure (II). Steric considerations would tend to favour structure **II**, but more conclusive evidence has been furnished by infrared spectrographic observations in Nujol mulls prepared from the dried propyl gallate salts of bismuth and antimony. The absorption spectra (see summarised results in Table II) confirm the absence of combined water and indicate the presence of free phenolic groups; this evidence favours structure **II.** The spectroscopic results also suggest that hydrogen bonding is present. This again is a most probable manifestation of the characteristics of structure **II.**



(I) (II)

EXPERIMENTAL

Examination of Table I suggests that propyl gallate will afford a ready means for separating and determining bismuth and tervalent antimony in alloys, pharmaceuticals, etc., containing zinc, cadmium, lead, copper and arsenic as impurities. Mercuric salts interfere, but mercurous salts give soluble gallate complexes. Bismuth may be precipitated as the n-propyl gallate complex from boiling 0·01 N nitric acid solutions; the yellow precipitate coagulates rapidly and permits immediate collection on a filter. The weight of the dried precipitate corresponds to the empirical formula $C_{10}H_{11}O_6.Bi$. Under more strongly acid conditions complete recovery may not be obtained.

Precipitates formed in the cold are finer and tend to clog the porous porcelain crucibles used for collection; precipitation from hot 0·01 N nitric acid solution is preferred because the precipitate can be rapidly collected, and this procedure has been consistently adopted for bismuth.

TABLE II

INFRARED SPECTRAL DATA FOR BISMUTH AND ANTIMONY PROPYL GALLATES

Wave number of peaks		
Bismuth	Antimony	Comments
3650	–	Free -OH stretching
3450	3255	Hydrogen bonded OH stretching
Bands between 2850 and 2950		CH_3—, CH_2— stretching (oil)
2680	2600	Chelate —OH stretching?
1686	1676	Ester C=O stretching
Bands between 1375 and 1640		Aromatic C—O stretching Phenolic C—O stretching —OH deformation CH_3—, CH_2— deformation (oil)
1342	1348	Phenolic —OH deformation C—O stretching
1253	1253	Ester C—O stretching
1218	1220	Phenolic —OH deformation C—O stretching
1156	1153	Ester C—O stretching
1087	1092	Ester C—O stretching?
1053	1073, 1035	—OH deformation?
Bands between 687 and 998		1,3,4,5 substituted benzene, out-of-plane deformation?

The effectiveness of this method for separating bismuth from several metals commonly associated with it in alloys has been examined. Table III indicates that the separation of bismuth from zinc, cadmium and copper is excellent, and spectrographic analysis of the ignited precipitates completely failed to detect cadmium and showed only a trace of copper. Lead, when present in a five-fold excess, does not significantly interfere with the determination of bismuth, but when present in fifty- or five-hundred-fold excess causes high results, and spectrographic analysis confirmed the presence of some lead in precipitates. Both antimony and tin are often present with bismuth in alloys and these elements interfere in the method, antimony^{III} forming an insoluble complex with propyl gallate and tin^{IV} and antimony^{V} hydrolysing to form insoluble hydrated oxides. Prior chemical separation of these cations is therefore necessary.

TABLE III

SEPARATION OF BISMUTH FROM OTHER CATIONS

Precipitations were carried out from solutions containing about 200 μg of bismuth per ml in 0·01 N nitric acid. Bismuth was weighed as $C_{10}H_{11}O_6.Bi$

Interfering element	Weight of interfering element, g	Bismuth added, mg	Bismuth recovered, mg	Error, %
—	—	49·76	49·46	−0·6
—	—	50·00	50·10	+0·2
—	—	49·88	50·00	+0·2
—	—	20·00	20·08	+0·4
—		21·13	21·13	0
Copper	2·5	50·10	50·01	−0·2
	2·5	50·00	49·98	0
	1·0	20·00	20·06	+0·3
	1·0	20·12	20·20	+0·4
Zinc	2·5	50·10	50·20	+0·2
	1·0	21·31	21·30	0
Cadmium	0·2	20·00	20·04	+0·2
	1·0	19·92	19·98	+0·3
Lead	2·5	50·12	50·52	+0·8
	1·0	20·00	20·18	+0·9
	1·0	19·99	20·06	+0·3
	0·25	50·61	50·31	−0·6

Conditions for the precipitation of antimony^{III} with propyl gallate are different from those for bismuth, as the antimony precipitate appears to be more soluble in hot solutions. Precipitation of antimony was therefore carried out at room temperature. Hydrochloric acid was chosen as the medium in this instance; it is to be preferred to nitric acid as a solvent for antimony trioxide because the formation of antimony^{V} is precluded. In 0·5 N hydrochloric acid a coarse white crystalline precipitate of the antimony^{III} - propyl gallate complex slowly forms after an initial inhibition period. When set aside overnight complete recovery of antimony is obtained, the weight of the dried precipitate corresponding to $C_{10}H_{11}O_6.Sb$. The separation of antimony from bismuth and arsenic^{III} has been examined and, as shown in Table IV, arsenic did not interfere. In the presence of bismuth, interference was sometimes observed, bismuth being detected spectrographically in the precipitate; the error sometimes is as high as 1 per cent.

TABLE IV

SEPARATION OF ANTIMONY FROM OTHER CATIONS

Precipitations were carried out from solutions containing about 200 μg of antimony per ml in 0·5 N hydrochloric acid

Interfering element	Weight of interfering element, mg	Antimony added, mg	Antimony recovered, mg	Error, %
—	—	20·00	20·06	+0·3
—	—	20·00	19·94	−0·3
Arsenic	100	20·00	20·12	+0·6
	200	40·52	40·58	+0·1
Bismuth	100	20·00	20·20	+1·0
	200	39·42	39·42	0·0

APPLICATIONS OF THE METHOD

In the practical application of this gravimetric procedure to determining bismuth in materials, it is often necessary to reduce the excess of acid by neutralisation with alkali. Bismuth is only metastable in solution at the acidity of precipitation (0·01 N nitric acid) and, on standing, slow hydrolysis takes place with deposition of bismuth compounds from solution as a fine white precipitate. We have found that the most satisfactory way of preventing complications owing to the precipitation of bismuth oxy-salts at low acid concentration is to condition the earlier stages of the analysis so that the final solution is approximately 0·2 N in nitric acid. The required acidity is then attained by twenty-fold dilution with water, the propyl gallate reagent then being added immediately to the boiling solution.

This procedure has potential applications in pharmaceutical and certain metallurgical fields of analysis. In the determination of bismuth and antimony in pharmaceuticals, organic matter can be destroyed by gentle ignition in an electric furnace, and the resulting trioxides can be dissolved in 2·5 N nitric acid for bismuth or 5·0 N hydrochloric acid for antimony before dilution to the appropriate acidity and precipitation of the propyl gallate complexes.

The bismuth content of a pharmaceutical whose base was predominantly magnesium carbonate - sodium hydrogen carbonate was found by the proposed method to be 0·65 per cent., compared with 0·59 per cent. by the normal phosphate method.

The method could, no doubt, be successfully applied to determining bismuth in alloys of the Woods metal type. These alloys contain tin, which must be removed by the procedure described below; similar alloys not containing tin or antimony may be dissolved in concentrated nitric acid. The excess of acid may then be neutralised by alkali in the manner described, or be removed by evaporation to dryness. When evaporation is used the residue is then dissolved in N nitric acid, diluted one-hundred-fold, and the bismuth is precipitated as its propyl gallate complex.

Tin and antimony frequently occur with bismuth in alloys, and when nitric acid is used to attack these alloys tin^{IV} and antimony^{V} are precipitated as their hydrated oxides. As tin is completely oxidised to the stannic form its removal from solution is quantitative; antimony, in the presence of a ten-fold excess of tin, is also completely removed from solution. This might serve as a method of separating these elements from bismuth, but we found that these hydrated oxides occluded appreciable amounts of bismuth. An alternative approach is to attack these alloys by a hydrobromic acid - bromine mixture and, after the addition of perchloric acid, to expel the volatile bromides of tin and antimony by evaporation to fumes of perchloric acid. It is necessary to repeat this process by the addition of hydrobromic acid alone, with subsequent evaporation, until all tin and antimony are removed, which is indicated by the final solution remaining clear. When this procedure is adopted, recovery of bismuth is good (see Table V).

TABLE V

SEPARATION OF TIN AND ANTIMONY FROM BISMUTH BY VOLATILISATION OF BROMIDES

The metals were dissolved in 10 ml of hydrobromic acid containing 15 per cent. v/v of bromine, 5 ml of perchloric acid were added, and the mixture was evaporated to fumes of perchloric acid. Then 5 ml of hydrobromic acid were added, and the mixture was again evaporated to fumes of perchloric acid. This operation was repeated until a clear solution was obtained. Traces of bromide were destroyed by the addition of a few drops of concentrated nitric acid and evaporation to fumes of perchloric acid

Interfering element	Weight of interfering element, mg	Bismuth added, mg	Bismuth recovered, mg	Error, %
—	—	50·17	50·14	−0·1
—	—	50·00	49·80	−0·4
Tin	500	50·00	49·80	−0·4
	500	50·00	49·88	−0·2
Antimony	500	50·00	49·85	−0·3

METHOD FOR DETERMINING BISMUTH

REAGENTS—

n-Propyl gallate solution—Dissolve 1 g of the reagent in 100 ml of warm water.
Wash solution—Nitric acid, 0·01 N.

PROCEDURE—

Bring 250 ml of 0·01 N nitric or perchloric acid solution containing about 50 mg of bismuth to the boil. Add slowly, with constant stirring, 25 ml of n-propyl gallate solution, bring the solution once more to the boil, and maintain at the boiling point for about one minute.

Filter the hot solution immediately, and collect the precipitate on a sintered-glass or porous-porcelain Gooch crucible. Wash the precipitate several times with the hot wash solution and finally once or twice with ethanol. Dry at 110° C for periods of one hour to constant weight.

100·0 mg of bismuth propyl gallate ≡ 47·91 mg of bismuth.

METHOD FOR DETERMINING ANTIMONY

REAGENTS—

n-Propyl gallate solution—Dissolve 1 g of the reagent in 100 ml of warm water.

Wash solution—Hydrochloric acid, 0·5 N.

PROCEDURE—

To a solution of antimony (about 40 mg) in 0·5 N hydrochloric acid add 25 ml of propyl gallate solution. Stir, and set aside at room temperature overnight.

Filter the cold solution through a porous-porcelain or sintered-glass Gooch crucible, and wash the precipitate several times with the cold wash solution, and finally once or twice with ethanol. Dry the precipitate at 110° C for periods of one hour to constant weight.

100·0 mg of antimony propyl gallate ≡ 34·89 mg of antimony.

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The Volumetric Determination of Iron, Molybdenum and Tungsten in Fluoride Solutions

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Both tungsten^{VI} and molybdenum^{VI} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid are quantitatively reduced to the tervalent state on a Jones reductor amalgamated to the extent of 0·75 per cent. w/w. They are determined, after collection in ammonium ferric sulphate solution, by titration with standard dichromate solution with barium diphenylamine sulphonate as indicator. Neither molybdenum^{VI} nor tungsten^{VI} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid is at all reduced on a silver reductor at 10° C, although iron^{III} is quantitatively reduced to iron^{II} under these conditions.

At 60° C in 0·2 M hydrofluoric acid - 1·5 to 2·0 M hydrochloric acid, molybdenum^{VI} is reduced quantitatively to molybdenum^{V} and in 0·2 M hydrofluoric acid - $>$ 4 M hydrochloric acid quantitatively to molybdenum^{III}. No reduction of tungsten^{VI} in 0·2 M hydrofluoric acid - hydrochloric acid occurs on a hot silver reductor at a hydrochloric acid concentration below 5·5 M.

Solutions containing iron^{III}, molybdenum^{VI} and tungsten^{VI} and no other species capable of reduction have, therefore, been analysed for all three metals by using the Jones and silver reductors.

The methods have been applied to the analysis of synthetic mixtures, a standard ferro-molybdenum alloy and a standard ferro-tungsten alloy. The mean determined compositions for the molybdenum and tungsten contents of the alloys were identical to the certificate compositions. The maximum deviation of any result from the mean determined compositions was 0·2 per cent.

It has already been reported[1] that tungsten^{VI} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid is quantitatively reduced to tungsten^{III} on passage through a Jones reductor (38 cm long, 1·8 cm internal diameter, packed with 16- to 30-mesh zinc shot amalgamated to the extent of 0·75 per cent. w/w). The tungsten^{III} solution was collected in ammonium ferric sulphate solution under oxygen-free nitrogen, and the equivalent amount of iron^{II} thus produced was titrated with standard potassium dichromate solution in the presence of phosphoric acid with barium diphenylamine sulphonate as indicator. Up to 18·4 mg of tungsten trioxide were determined with a mean error for 16 determinations of − 0·00(3) mg and a standard deviation from the mean error of 0·02(1) mg.

For amounts of tungsten trioxide in excess of 18·4 mg the reduction is not quantitative. This incomplete reduction is certainly associated with the fact that the tungsten^{III} species is fairly rapidly oxidised by hydrogen ion.[1] In an effort to extend the upper limit for quantitative reduction of tungsten^{VI}, the effect of different degrees of amalgamation of the zinc was investigated. A plot of oxidation state of the tungsten in the effluent from the reductor against amount of tungsten trioxide taken is shown in Fig. 1.

It is obvious that an amalgamation of 0·75 per cent. w/w, which was originally chosen, produces the most satisfactory conditions for reduction.

As expected, iron^{III} and molybdenum^{VI} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid are quantitatively reduced to iron^{II} and molybdenum^{III} under the same conditions as used for tungsten^{VI}. Up to 15·7 mg of iron^{III} were determined with a mean error for 11 determinations of —0·00(3) mg and a standard deviation from the mean error of 0·02(0) mg.

Up to 20·3 mg of molybdenum trioxide were determined with a mean error for 13 determinations of —0·00(5) mg and a standard deviation from the mean error of 0·01(8) mg.[1] In view of the fact that molybdenum^{III}, in contrast to tungsten^{III}, is much less rapidly oxidised by hydrogen ion,[1] it was surprising to find that the upper limit for the quantitative determination of molybdenum trioxide was 20·3 mg. On further investigation, it was ascertained that low results for amounts of molybdenum trioxide in excess of 20·3 mg were caused not by incomplete reduction to molybdenum^{III}, but by premature end-points in the subsequent titration. Molybdenum^{III} from the reductor reacts with iron^{III} in the collecting vessel to produce iron^{II} and molybdenum^{V}. The iron^{II} reacts rapidly with dichromate in the titration, but molybdenum^{V} reacts only sluggishly, and a premature end-point is obtained for amounts of molybdenum trioxide in excess of 20·3 mg. For these larger amounts of molybdenum the indicator is oxidised before all of the molybdenum^{V} is titrated. By using the E.E.L. titrator with an Ilford No. 601 filter for photometric end-point detection, it was established that the molybdenum^{VI} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid is, in fact, quantitatively reduced to molybdenum^{III} for amounts up to at least 50·8 mg. The photometric titrations of iron^{II} *plus* molybdenum^{V} were made with standard dichromate solution in the absence of indicator. The end-point occurred at the intersection of two extrapolated straight lines on the plot of relative optical density against volume of titrant added, but the fact that the galvanometer needle took some time to fall to a steady value in the vicinity of the end-point verified that the reaction between molybdenum^{V} and dichromate was indeed slow.

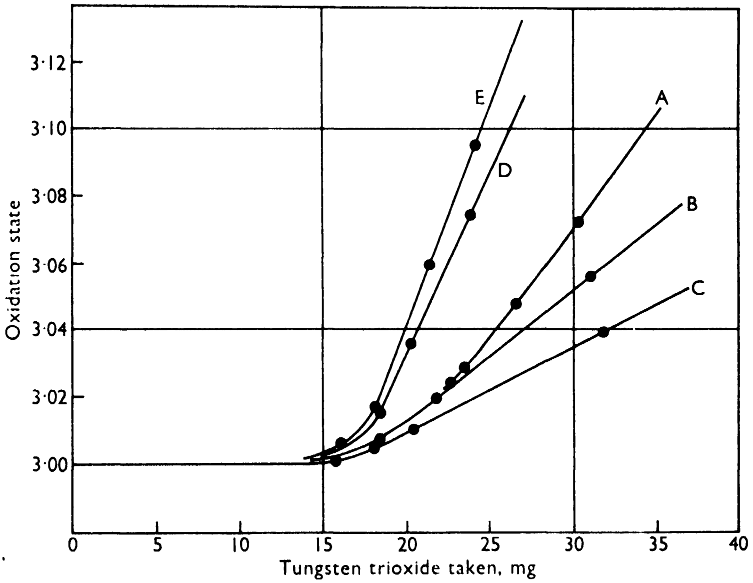

Fig. 1. Oxidation state of tungsten in the effluent from the Jones reductor as a function of the weight of tungsten trioxide taken for degrees of amalgamation of: curve A, 0·1 per cent. w/w; curve B, 0·5 per cent. w/w; curve C, 0·75 per cent. w/w; curve D, 1·0 per cent. w/w; curve E, 1·5 per cent. w/w

Although amounts of molybdenum trioxide between 20·3 and 50·8 mg could certainly be determined quantitatively by photometric titration, the method is not recommended because it is much slower than a visual titration. Conditions are easily arranged so that no more than 15 to 20 mg of molybdenum trioxide are passed through the reductor.

Incidentally, the low recoveries for amounts of tungsten trioxide in excess of 18·4 mg are associated with the side-reaction of tungsten^{III} and hydrogen ion in the reductor, and not with the titration. A photometric titration with dichromate of amounts of tungsten trioxide in excess of 18·4 mg after reduction and collection in iron^{III} solution, and in the absence of indicator, also produced low results. Iron^{III} and tungsten^{III} appear to react to give iron^{II} and tungsten^{VI}, and iron^{II} is titrated quantitatively with dichromate before the indicator is oxidised.

Although the method described above for the volumetric determination of tungsten is completely satisfactory, tungsten is often associated with iron and molybdenum, and it was felt that the full potentialities of the method would only be realised if suitable volumetric procedures that would lead to a quantitative determination of all three elements in mixtures could be developed.

It was therefore decided to investigate the reduction of iron^{III}, molybdenum^{VI} and tungsten^{VI} in fluoride solutions, on the silver reductor. It was soon established that iron^{III} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid, at about 10° C, is quantitatively reduced to iron^{II} on the silver reductor, whereas molybdenum^{VI} and tungsten^{VI} are unaffected. The reduced solution was again collected in ammonium ferric sulphate solution under nitrogen, and the iron^{II} was titrated with dichromate in the presence of phosphoric acid and barium diphenylamine sulphonate. Up to 22·0 mg of iron^{III}, alone and in the presence of molybdenum^{VI} and tungsten^{VI}, have been determined with a maximum error of 0·04 mg.

The fact that molybdenum^{VI} is not reduced at all in this medium is of considerable interest, because molybdenum^{VI} in 2 M hydrochloric acid is partly reduced to molybdenum^{V} at about 10° C on passage through the silver reductor (extent of reduction approximately 55 per cent.). It is evident that the molybdenum^{VI} is stabilised in the presence of hydrofluoric acid and is less easily reduced. Molybdenum^{VI} can, of course, be reduced quantitatively in solutions of 2 M hydrochloric acid at 60° to 80° C on passage through a silver reductor,[2] and experiments were undertaken with a hot silver reductor to see if molybdenum^{VI} in hydrochloric - hydrofluoric acid solutions could be reduced to a definite oxidation state. Because hydrofluoric acid inhibits the reduction of molybdenum^{VI}, the concentration of this acid was reduced to 0·2 M, which is still sufficient to retain tungsten^{VI} in true solution. The extents of reduction of both molybdenum^{VI} and tungsten^{VI} in 0·2 M hydrofluoric acid containing 0·5 to 7 M hydrochloric acid are shown in Fig. 2.

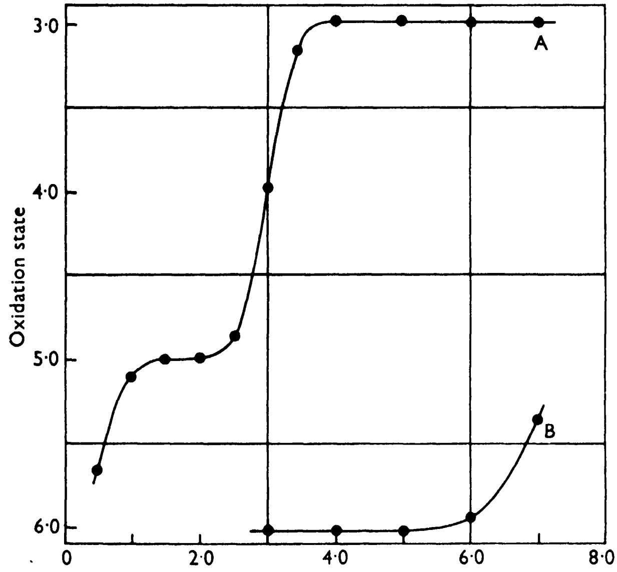

Fig. 2. Oxidation state of molybdenum and tungsten in the effluent from the hot silver reductor as a function of hydrochloric acid concentration: curve A, molybdenum; curve B, tungsten. The effluent was also 0·2 M in hydrofluoric acid.

As can be seen, molybdenum^{VI} is quantitatively reduced to molybdenum^{V} in 0·2 M hydrofluoric acid - 1·5 to 2·0 M hydrochloric acid, and to molybdenum^{III} in 0·2 M hydrofluoric acid - >4 M hydrochloric acid. Tungsten^{VI} is not reduced until the hydrochloric acid concentration exceeds 5·5 M.

By using synthetic solutions, it was established that up to 13·2 mg of molybdenum trioxide in 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid, both in the presence and absence of tungsten^{VI}, could be determined on the hot silver reductor with a maximum error of 0·04 mg. Iron^{III}, as expected, is quantitatively reduced to iron^{II} under these conditions.

The scheme outlined below is, therefore, available for determining mixtures of iron^{III}, molybdenum^{VI} and tungsten^{VI}. Iron^{III}, alone, is reduced on the cold silver reductor in 2 M hydrochloric acid - 0·5 M hydrofluoric acid. Iron^{III} and molybdenum^{VI} are reduced together on the hot silver reductor in 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid. Iron^{III}, molybdenum^{VI} and tungsten^{VI} are all quantitatively reduced on the Jones reductor in 2 M hydrochloric acid - 0·5 M hydrofluoric acid. The results obtained for the analysis of synthetic mixtures of iron^{III}, molybdenum^{VI} and tungsten^{VI}, a ferro-molybdenum alloy and a ferro-tungsten alloy are reported below.

METHOD

APPARATUS—

Because of the corrosive nature of hydrofluoric acid solutions towards glassware, the apparatus used was constructed entirely in polythene.

The Jones reductor was prepared from heavy-gauge polythene tubing (length 38 cm; internal diameter 1·80 cm) narrowing rapidly at the base to a short piece of narrow-bore tubing, 4 mm in diameter. A polythene funnel 1½ inches in diameter at the maximum width, welded to the base of the reductor, served this purpose. The zinc shot was supported by a perforated disc of diameter 6 mm made from ⅛-inch polythene sheet and inserted into the wider end of the polythene funnel stem.

Polythene tubing (6 mm bore) was wrapped round the column from top to bottom in the form of a spiral, for cooling purposes. The collecting vessel was a 250-ml polythene bottle fitted with a polythene screw-cap having three holes in the top to accommodate the column delivery tube, and inlet and outlet tubes for nitrogen. The inlet tube reaches almost to the bottom of the bottle. A solution reservoir at the top of the reductor was made by welding a polythene funnel (3½ inches in diameter at the maximum width), from which the stem had been removed at the appropriate position, on to the top of the column. A screw-clamp at the base of the reductor prevented leakage when the column was not in use. The column of amalgamated zinc was 38 cm long.

The silver reductor was prepared from polythene tubing (length 38 cm; internal diameter 1·0 cm) narrowing rapidly at the base to a short piece of narrow-bore tubing, 5 mm in diameter, just long enough to protrude into the collecting vessel. The central portion (28 cm long) of the reductor tube was encased in heavy-gauge polythene tubing (1·8 cm internal diameter) sealed on to the reductor at each end, with an inlet tube at the bottom and an outlet tube at the top, in the form of a Liebig condenser. This permitted the temperature of the reductor to be controlled as required. The solution reservoir at the top of the reductor, the perforated disc for supporting the silver, and the collecting bottle were constructed and fitted to the column as for the Jones reductor. The column of silver was 34 cm long.

REAGENTS—

Hydrofluoric acid and other acids—These were of analytical-reagent grade.

Nitrogen—This was oxygen-free "spot" nitrogen obtained from the British Oxygen Co. Ltd.

Potassium dichromate solution—An exactly 0·1000 N (M/60) solution of potassium dichromate was prepared from the analytical-reagent grade salt.

Oxygen-free distilled water—This was prepared by boiling distilled water vigorously for 15 minutes and allowing it to cool under liquid paraffin. The water was stored under liquid paraffin.

Standard solutions of iron, molybdenum and tungsten—(1) *Iron*—This was prepared from AnalaR ammonium ferric sulphate that had been analysed for iron by using the usual gravimetric method of hydroxide precipitation, and then filtration and ignition to ferric oxide. (2) *Molybdenum and tungsten*—These were prepared from Specpure trioxides, obtained from Johnson, Matthey and Co. Ltd., by dissolving accurately weighed amounts of the trioxides in boiling ammonium hydroxide, sp.gr. 0·88, in 100-ml polytetrafluoroethylene beakers. When dissolution was complete the solutions were evaporated to dryness to remove the excess of ammonia. The resulting solids were dissolved by heating gently with 10 ml of a solution of diluted hydrofluoric acid (2 + 3), adjusted to be 0·5 M in hydrofluoric acid and stored in screw-cap polythene bottles. The concentration of the element was arranged to be approximately 3 to 4 mg per g of solution.

PREPARATION OF AMALGAMATED ZINC SHOT—

AnalaR zinc shot, 16 to 30 mesh (batch Nos. 53324 and 58066), was obtained from Hopkin and Williams Ltd. The zinc shot was amalgamated to the extent of 0·75 per cent. w/w by vigorously shaking 500 g of shot—previously washed with dilute hydrochloric acid

(2 + 98) and then with distilled water—with 250 ml of 2 per cent. w/v mercuric chloride solution in a stoppered flask for 45 to 60 seconds. The supernatant solution was discarded, and the shot was washed several times with distilled water and then once with dilute sulphuric acid (2 + 98). The shot was finally washed with distilled water and stored under dilute hydrochloric acid (1 + 99).

Zinc shot amalgamated to the extent of 0·1 per cent., 0·5 per cent., 1·0 per cent. or 1·5 per cent. w/w was prepared in the same way by shaking, in each instance, 500 g of shot with the appropriate amount of mercuric chloride solution.

PREPARATION OF SILVER FOR THE SILVER REDUCTOR—

The silver was prepared from AnalaR silver nitrate by dissolving 40 g of the salt in 250 ml of distilled water containing 5 ml of nitric acid, sp.gr. 1·42. This solution was then agitated with a sheet of electrolytic copper until deposition of the silver was complete. The precipitated silver was washed thoroughly with dilute sulphuric acid (1 + 99) until free from copper^{II} ions, and then with distilled water until free from sulphuric acid. The silver was stored under dilute hydrochloric acid (1 + 99).

PREPARATION OF THE FERRO ALLOYS FOR ANALYSIS—

Dissolve 0·1 to 0·2 g of alloy in 5 ml of 40 per cent. w/w hydrofluoric acid and 1 ml of 36 per cent. w/w hydrochloric acid in a 100-ml polytetrafluoroethylene beaker. Add 1 ml of 100-volume hydrogen peroxide, and heat gently until dissolution is complete.

Evaporate the solution gently to dryness, and redissolve the solid in 5 ml of 40 per cent. w/w hydrofluoric acid. Repeat this operation twice to destroy excess of hydrogen peroxide. Redissolve the solid in 5 ml of diluted hydrofluoric acid (2 + 3), and transfer the solution to a dry, pre-weighed, 100-ml polythene bottle fitted with a polythene screw-cap, washing the beaker several times with oxygen-free distilled water. Transfer the washings to the bottle.

Place 5 ml of dilute hydrofluoric acid (1 + 10) in the beaker, and boil gently for a few minutes. Transfer the solution and subsequent beaker washings to the bottle, and dilute to a suitable volume with oxygen-free distilled water. Cool the bottle and solution to room temperature, and re-weigh.

PROCEDURES FOR DETERMINING IRON^{III}, MOLYBDENUM^{VI} AND TUNGSTEN^{VI}—

(*a*) *Iron*—Transfer a suitable weighed portion of solution containing not more than 22·0 mg of iron to a 65-ml (2-oz) polythene bottle calibrated at 50 ml. Add sufficient 36 per cent. w/w hydrochloric acid and dilute hydrofluoric acid (1 + 9) so that the final composition of the solution, when diluted to 50 ml with oxygen-free distilled water, is 2·0 M in hydrochloric acid and 0·5 M in hydrofluoric acid. Dilute to the 50-ml mark.

Connect the water-jacket of the silver reductor to a cold-water tap, and allow water to pass through freely. Then open the screw clamp on the bottom of the reductor column. Allow the solution level inside the reductor to fall almost to the silver, and add 25 ml of 2 M hydrochloric acid - 0·5 M hydrofluoric acid wash solution to the reductor reservoir. Pass this solution through the reductor at a flow rate of 5 ml per minute. When the solution level again almost reaches the silver add a further 25-ml portion. Repeat this operation until 100 ml of wash solution have been passed through the reductor. At no time should the solution level fall below the level of the silver.

When the final 25-ml portion of wash solution has been added to the reservoir, start the flow of nitrogen to the inlet tube of the collecting bottle, and adjust the flow-rate to approximately 250 ml per minute.

Immediately before the level of solution in the reductor reaches the silver, attach to the base of the column a 250-ml polythene bottle containing 10 ml of 0·1 M ammonium ferric sulphate solution and 30 ml of phosphoric acid, sp.gr. 1·75.

Introduce the iron^{III} solution into the top of the column in 5- to 10-ml portions, and maintain a constant depth of solution above the silver. Take 50 ml of 2 M hydrochloric acid - 0·5 M hydrofluoric acid wash solution, and rinse the 2-oz polythene bottle with three 5-ml portions. Pour these rinsings on to the column, allowing the level of solution in the column to fall almost to the level of the silver before adding the next portion. Add the remaining 35 ml of wash solution in a similar manner. When the level of the final 5-ml portion has almost reached the silver, unscrew the collecting vessel, and rinse the nitrogen-flow tube with oxygen-free distilled water during removal of the bottle.

Add 30 ml of phosphoric acid, sp.gr. 1·75 (see Note 1), and 10 ml of sulphuric acid, sp.gr. 1·84. Dilute the solution to 230 to 240 ml with oxygen-free distilled water. Add exactly 5 drops (0·25 ml) of an aqueous 0·2 per cent. w/v solution of barium diphenylamine sulphonate as indicator, and titrate the solution with 0·1000 N potassium dichromate to a permanent purple end-point. Add titrant from a 5- or 10-ml calibrated grade-A micro-burette. Subtract from the titre a blank value (see Note 2 (*i*)).

Process a duplicate portion of iron solution of approximately half or double the weight of the first portion.

1·000 ml of 0·1000 N potassium dichromate ≡ 5·585 mg of iron.

(*b*) *Iron and molybdenum*—Transfer a suitable weighed portion of solution containing not more than 22·0 mg of iron and 13·2 mg of molybdenum trioxide (8·8 mg of molybdenum) to a 65-ml (2-oz) polythene bottle calibrated at 50 ml. Add sufficient 36 per cent. w/w hydrochloric acid and dilute hydrofluoric acid (1 + 49) so that the final composition of the solution, when diluted to 50 ml, is 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid. Dilute the solution to approximately 40 ml with distilled water, and pass a steady stream of oxygen-free nitrogen through it for 15 minutes (see Note 2 (*ii*)). Rinse the nitrogen-flow tube with oxygen-free distilled water during removal of the bottle, and dilute the solution to the 50-ml mark.

Connect the water-jacket of the silver reductor to a supply of water at 70° C (see Note 3), and allow the temperature of the reductor to become constant. Then open the screw-clamp at the base of the reductor. When the level of solution in the reductor column has almost fallen to the silver, add 25 ml of 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid wash solution, and wash the column in the same way as with the iron solution in procedure (*a*), but use 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid wash solution.

When the final 25-ml portion of wash solution has been added to the reservoir, heat the sample solution by immersing its container in a small reservoir into which is flowing the waste water from the reductor heater. The reservoir was constructed from a 250-ml polythene bottle, from which the top part had been removed at the appropriate position. A side-arm was fitted to the reservoir ¼ inch from the top as an overflow pipe.

When the level of the final 25-ml portion of wash solution has fallen almost to the level of the silver, attach a collecting bottle containing 10 ml of 0·1 M ammonium ferric sulphate solution and 30 ml of phosphoric acid, sp.gr. 1·75, to the base of the reductor. Introduce the hot solution containing the iron and molybdenum into the top of the reductor in 5- to 10-ml portions, and process as in procedure (*a*), but use 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid wash solution.

Rinse the nitrogen-flow tube with oxygen-free distilled water during the removal of the collecting bottle. Add 30 ml of phosphoric acid, sp.gr. 1·75 (see Note 1), and dilute the solution to 230 to 240 ml with oxygen-free distilled water. Add indicator, and titrate the solution as in procedure (*a*). Subtract from the titre a blank value (see Note 2 (*ii*)) and a volume of 0·1000 N potassium dichromate equivalent to the amount of iron present in the portion.

Process a duplicate portion of approximately half or double the weight of the first.

1·000 ml of 0·1000 N potassium dichromate
≡ 4·798 mg of molybdenum trioxide
≡ 3·198 mg of molybdenum.

(*c*) *Iron, molybdenum and tungsten*—Transfer a suitable weighed portion of solution containing not more than 15·7 mg of iron, 20·3 mg of molybdenum trioxide (13·5 mg of molybdenum) and 18·4 mg of tungsten trioxide (14·6 mg of tungsten) to a 65-ml (2-oz) polythene bottle calibrated at 50 ml. Add sufficient 36 per cent. w/w hydrochloric acid and dilute hydrofluoric acid (1 + 9) so that the final composition of the solution, when diluted to 50 ml with oxygen-free distilled water, is 2 M hydrochloric acid - 0·5 M hydrofluoric acid. Dilute to the 50-ml mark.

Connect the cooling-spiral of the Jones reductor to a cold-water tap, and run the water for some minutes. Then open the screw-clamp at the base of the reductor. Drain the solution in the column until the level almost reaches the zinc shot, and wash the column with four 25-ml portions of 2 M hydrochloric acid - 0·5 M hydrofluoric acid wash solution.

Process the sample solution in exactly the same way as described in procedure (*a*), but use 35 ml of wash solution instead of 50 ml to wash the sample solution through the reductor.

Rinse the nitrogen-flow tube with oxygen-free distilled water during removal of the collecting vessel.

Add 30 ml of phosphoric acid, sp.gr. 1·75 (see Note 1), and 10 ml of sulphuric acid, sp.gr. 1·84. Dilute the solution to 230 to 240 ml with oxygen-free distilled water. Add indicator solution, and titrate exactly as in procedure (*a*).

Subtract from the titre a blank value (see Note 2 (*iii*)) and calculated volumes of 0·1000 N potassium dichromate equivalent to the amounts of iron and molybdenum present in the portion of sample solution.

1·000 ml of 0·1000 N potassium dichromate
≡ 7·730 mg of tungsten trioxide
≡ 6·131 mg of tungsten.

NOTES

1. INTERFERENCE FROM TUNGSTEN^{VI}—

It has already been reported[3] that the end-point as shown by the colour change of barium diphenylamine sulphonate indicator in the titration of iron^{II} with potassium dichromate solution, is retarded in the presence of chloride ion. This effect increases with increasing concentration of chloride ion in the titrand, but is constant for a fixed concentration of chloride ion. The chloride ion concentration is therefore partly responsible for the magnitude of the blank value.

However, the blank value was found to vary when iron^{II}, in the presence of a fixed concentration of chloride ion but increasing amounts of tungsten^{VI}, was titrated with potassium dichromate with barium diphenylamine sulphonate as indicator. The blank value decreased as the concentration of tungsten^{VI} increased. Tungsten^{VI} appears to have a catalytic effect on the colour change of the indicator. Why chloride ion and tungsten^{VI} should influence the oxidation mechanism of the indicator is not obvious, but the variable effect produced by different amounts of tungsten^{VI} can be overcome by adding 30 ml of phosphoric acid, sp.gr. 1·75 (making 60 ml in all), before titrating the iron^{II} with dichromate. Under these conditions the blank value is constant in the presence of a fixed amount of chloride ion and 0 to 60 mg of tungsten trioxide.

2. DETERMINATION OF BLANK VALUES—

(*i*) *The cold silver reductor*—There are four sources of error, three positive and one negative. These are (*a*) impurities present in the reagents, (*b*) chloride ion concentration, (*c*) theoretical indicator blank value—all positive effects—and (*d*) hydrogen peroxide produced in the silver reductor[4] by reduction of dissolved oxygen present in the solutions—a negative effect. The positive effects are constant for a fixed chloride ion concentration, but the negative effect may differ from one solution to another according to oxygen content.

Because a sample solution, whose iron content is to be determined, may have a different oxygen content from that of a standard iron solution, it is not advisable to apply to the sample solution a correction for a blank value determined for a standard solution. The blank value should be obtained by processing two portions of sample solution, which should be fairly different in weight, and applying the equation—

$$b = \frac{W_1V_2 - W_2V_1}{W_1 - W_2}$$

where b is the blank value in millilitres, W_1 and W_2 are weights of sample solution taken and V_1 and V_2 are the titres in millilitres for W_1 and W_2, respectively.

(*ii*) *The hot silver reductor*—Although the blank value is reasonably constant for various amounts of standard iron^{III} solution passed through a cold silver reductor, this is not so for a hot silver reductor. The blank value is generally lower, presumably because more hydrogen peroxide is produced, and is more variable. Since different portions of a sample solution, after adjustment to a volume of 50 ml with hydrochloric and hydrofluoric acids, are unlikely to have identical oxygen contents, it was considered advisable to remove the oxygen from these solutions by passing oxygen-free nitrogen through them for 15 minutes before processing them on the reductor.

Various amounts of iron^{III} solution, which had been gassed out with nitrogen and passed through the hot reductor, showed, as expected, a higher and much more consistent blank value. The blank values are again determined by using the equation shown in Note 2 (*i*).

(*iii*) *The Jones reductor*—No difficulty from hydrogen peroxide is encountered in using the Jones reductor, since any oxygen present in the solution being passed through the reductor is completely reduced to water.[5] To determine the blank value for the Jones reductor, process a series of various standard amounts of iron^{III} in 2 M hydrochloric acid - 0·5 M hydrofluoric acid. (Molybdenum^{VI} or tungsten^{VI} can also be used, but iron^{III} is cheapest.) In each determination calculate the volume of 0·1000 N potassium dichromate theoretically required to titrate the iron^{II} produced by the reductor, and subtract this volume from the experimental titre. None of the blank values should differ from the mean blank value by more than 0·004 ml.

A typical blank value for the Jones reductor for an iron^{III} solution was 0·105 ml.

3. HOT WATER SUPPLY—

The temperature of the water supply to the silver reductor was maintained at 70° C by passing warm water at 40° C through two Liebig condensers connected in series, up the centre of which steam was passing. The temperature of the water supply to the reductor could then be adjusted by altering either the rate of flow of the water or the pressure of steam. The fall in temperature from the bottom to the top of the reductor column is 3° to 4° C.

The temperature of the effluent containing the reduced species under these conditions is 58° to 60° C.

RESULTS

TABLE I

ANALYSIS OF SOLUTIONS OF PREPARED MIXTURES OF IRON, MOLYBDENUM AND TUNGSTEN

Solution number	Iron content		Molybdenum trioxide content		Tungsten trioxide content	
	Actual, mg	Determined, mg	Actual, mg	Determined, mg	Actual, mg	Determined, mg
1	32·5	32·6	74·2	74·2	20·5	20·6
2	30·6	30·6	30·9	30·7	73·4	73·5
3	46·2	46·2	47·7	47·8	52·3	52·3
4	68·0	68·1	35·8	35·9	74·1	74·0
5	63·1	63·0	74·7	74·9	18·2	18·2

Solutions were prepared from stock solutions of the three elements; the total weight of each solution was approximately 30 g. Suitable fractions were analysed by the methods described above, and the results are shown in Table I.

TABLE II

RESULTS FOR THE ANALYSIS OF FERRO ALLOYS

Alloy		Sample number	Determined composition		
B.C.S. number	Certificate composition, %		Iron, %	Molybdenum, %	Tungsten, %
231/2	Molybdenum 70·1	1	28·8, 28·9	70·2, 70·0, 70·2,* 70·0,* 69·9,* 70·2*	—
		2	28·9, 29·0	70·0, 70·0, 70·1,* 70·2,* 69·9*	—
242/1	Tungsten 82·0	1	17·5, 17·5	0·3(2), 0·3(2)	82·0, 82·0
		2	17·4, 17·6	0·3(5), 0·3(5)	82·0, 81·9

* Obtained by using the Jones reductor; the other molybdenum values were obtained by using the silver reductor.

DISCUSSION

Although the maximum amount of molybdenum^{VI} in 4·5 M hydrochloric acid - 0·2 M hydrofluoric acid, determined by using the hot silver reductor, was 13·2 mg expressed as molybdenum trioxide (8·8 mg of molybdenum), it is extremely likely that, as with the Jones reductor, amounts of molybdenum trioxide up to 20·3 mg (13·5 mg of molybdenum) could be determined, by using a visual titration with potassium dichromate solution and barium diphenylamine sulphonate as indicator.

The maximum amount of iron^{III} determined in 2 M hydrochloric acid - 0·5 M hydrofluoric acid by using the Jones reductor was 15·7 mg, but much larger amounts are likely to be quantitatively reduced on this reductor.

It is evident from Table II that the methods just described should be useful for determining iron, molybdenum and tungsten in ferro-molybdenum, ferro-tungsten and certain other alloys, preferably after a qualitative spectrographic analysis to make absolutely certain that the alloys contain no appreciable amounts of other elements that could interfere in the methods by being reduced on the silver or Jones reductors.

The behaviour of a few elements that can be quantitatively reduced to lower oxidation states on metal reductors from solutions free from fluoride was investigated for the fluoride systems employed for iron, molybdenum and tungsten. In all instances the maximum amount of element passed through the reductor was 14·3 mg. The procedures used for the reductions and titrations were identical to those employed for iron, molybdenum and tungsten. The results are shown in Table III.

TABLE III

OXIDATION STATES OF VANADIUM, TITANIUM AND CHROMIUM AFTER PASSAGE THROUGH VARIOUS REDUCTORS

Species	Oxidation states of the species in the effluent from the reductor		
	Cold silver	Hot silver	Jones
Vanadium^{V}	+4·00	+4·00	+2·00
Titanium^{IV}	+4·00	+4·00	+3·00
Chromium^{III}	+3·00	+3·00	+2·83

Chromium^{III} is not reduced to a definite oxidation state on the Jones reductor in cold 2 M hydrochloric acid - 0·5 M hydrofluoric acid.

Vanadium^{II} reacts with iron^{III} in the collecting vessel to produce iron^{II} and vanadium^{IV}. In the titration of such a solution with barium diphenylamine sulphonate as indicator, the indicator changes colour before any of the vanadium^{IV} is oxidised. Vanadium^{IV} can, however, be determined by photometric titration with dichromate in the absence of the indicator.

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The Volumetric Determination of Antimony in Antimony-Lead and Antimony-Tin-Lead Alloys

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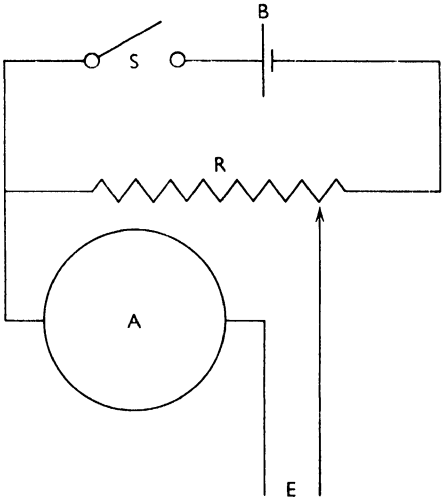
A method is described for determining antimony by titration with bromate involving the use of the dead-stop end-point. The proposed method has advantages over the indicator method and has been successfully applied to the determination of antimony in lead - antimony and tin - lead - antimony alloys. Up to 10 mg of copper and 50 mg of iron can be tolerated without seriously affecting the results.

POTASSIUM bromate is widely used for the titration of antimony,[1,2,3] but the methyl orange indicator commonly employed is non-reversible, is subject to fading and introduces a blank titre that varies with the amount of indicator added. Reversible indicators, which are an improvement over methyl orange, have been reported by Belcher.[4,5] An amperometric titration method has been described by Harris and Lindsey[6] for determining antimony with potassium bromate, a vibrating platinum micro-electrode being used.



A = Microammeter, 0 to 100 μA.
B = Dry cell, 1·5 volts.
E = Electrodes (5-cm lengths of 24 s.w.g. platinum wire)
R = 200-ohm wire-wound radio-type potentiometer.
S = Single-pole ON-OFF switch.

Fig. 1. Circuit diagram

This paper deals with the satisfactory application of the dead-stop end-point to the titration of antimony. First described by Foulk and Bawden,[7] the dead-stop end-point has since found many applications. In the proposed method a potential is applied across a pair of platinum electrodes immersed in the solution to be titrated, and a microammeter measures the current flowing between the electrodes. When tervalent antimony is titrated in this apparatus, the cell is polarised and the current flowing remains at a low level until the whole of the antimony is oxidised to the quinquivalent state; a slight excess of bromate, which acts as a depolariser, is then sufficient to cause a large deflection of the microammeter. Details of the circuit are shown in Fig. 1.

EXPERIMENTAL

CHOICE OF POLARISING POTENTIAL—

To determine the optimum polarising potential, solutions containing 5 ml of 0·1 N antimony solution, 5 ml of concentrated sulphuric acid and 40 ml of concentrated hydrochloric acid were diluted to 300 ml, and the stirred solutions were titrated at 80° C with 0·1 N potassium bromate, various applied potentials being used. Results are shown graphically in Fig. 2. Changes in the applied potential between 0·2 and 0·6 volt cause little change in sensitivity, but the residual current increases rapidly with potential. For this reason a potential of 0·2 volt was chosen.

WARNING OF THE APPROACH OF THE END-POINT—

In the earlier work with the method when the electrodes were positioned well into the solution there appeared to be no warning of the approach of the end-point, and an excess of titrant was easily added unless the titration was carried out dropwise. It was found, however, that a warning could be obtained by positioning the electrodes close to the surface of the solution, with the cathode near to the point of entry of the titrant. At the start of the titration the bromate is consumed rapidly, and the needle of the microammeter remains almost stationary. As the titration proceeds, local concentrations of bromate build up near the point of entry of the titrant, depolarise the cathode and cause oscillations of the microammeter needle, which become more violent as the end-point is neared. At the end-point, when a slight excess of bromate exists throughout the solution, a permanent deflection is obtained.

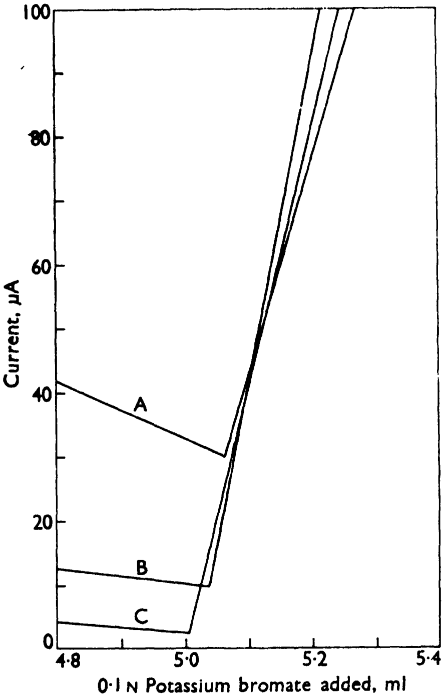


Fig. 2. Determination of optimum polarising potential: curve A, 0·6 volts; curve B, 0·4 volts; curve C, 0·2 volts

EFFECT OF TEMPERATURE—

When titrations are carried out at room temperature the response of the meter is slow, and consequently the end-point is easily overshot. At intermediate temperatures, *i.e.*, about 40° C, the microammeter needle oscillates with each addition of bromate, making it difficult to judge when the titration is nearing completion. If, however, the solution is titrated between 80° and 90° C only slight oscillations take place until within a few millilitres of the end-point; then the oscillations increase in amplitude reaching almost full-scale deflection within a few drops of the end-point.

EFFECT OF VARIOUS IONS—

To test the effect of various ions they were added to a solution of known antimony content. After the addition of 75 ml of concentrated hydrochloric acid and 1 ml of saturated sulphur dioxide solution the mixture was evaporated, with vigorous boiling, to 60 ± 5 ml, diluted to 300 ml, and titrated at a temperature between 80° and 90° C with 0·1 N potassium bromate.

Tin and lead—To test the effect of tin and lead, the metals were dissolved by heating in sulphuric acid.

Up to 2·5 g of tin and 5 g of lead did not cause any interference, although it should be noted that with amounts of lead greater than about 2 g crystallisation occurs during the evaporation, and this gives rise to serious superheating unless the beaker is continually agitated.

Copper—Because of the possibility of the method being used for determining antimony in white metals, the effect of copper was tested. Table I shows the results obtained in the presence of copper. Amounts above 10 mg cause serious interference and, therefore, if more than 10 mg of copper are present a separation is necessary.

TABLE I

EFFECT OF COPPER

Copper added, mg	Antimony added, mg	Antimony found, mg
10	60·9	60·3
50	60·9	59·2
100	60·9	58·1
150	60·9	57·5
50	150·2	147·8
100	150·2	146·2
150	150·2	145·6

Arsenic—The evaporation stage in the method satisfactorily removes up to 10 mg of arsenic, but, as expected, any arsenic remaining is titrated by the bromate and gives high results. The response of arsenic, titrated under the same conditions as outlined under "Method," is similar to that of antimony, and the proposed method has been used in this laboratory for determining arsenic in certain materials.

Iron—In lead - antimony alloys only extremely small amounts of iron would be normally encountered, but the effect of iron, added in the ferric form, was tested because it is an element likely to cause difficulty when an indicator is used. The effect of iron on the titration is to produce a high residual current that drops rapidly just before the end-point is reached. Thus, at the beginning of the titration a solution containing 100 mg of iron had a residual current of 60 μA and, when near to the end-point, this fell to 10 μA. Results in Table II show that iron causes slightly low results, but amounts of up to 50 mg have no significant effect.

TABLE II

EFFECT OF IRON

Iron added, mg	Antimony added, mg	Antimony found, mg
25	60·9	60·6
50	60·9	60·4
100	60·9	59·5
25	150·2	149·5
50	150·2	149·0
100	150·2	148·2

Other ions—Five grams of ammonium or potassium sulphate, used to facilitate the dissolution of the sample by elevating the boiling-point of the sulphuric acid, caused no interference.

METHOD

REAGENTS—

Sulphuric acid, concentrated.

Potassium sulphate.

Hydrochloric acid, concentrated.

Sulphur dioxide, saturated solution.

Potassium bromate, 0·1 N, *aqueous*—Dissolve 2·784 g of potassium bromate in 1 litre of water, and standardise against pure antimony.

PROCEDURE—

Weigh accurately 0·25 to 2 g of sample according to the antimony content, transfer to a 400-ml beaker, and add 10 ml of concentrated sulphuric acid and 5 g of potassium sulphate. Heat gently to dissolve the sample, and then heat over an open flame to expel any sulphur. Cool, and then add 10 ml of water, 75 ml of concentrated hydrochloric acid and 1 ml of saturated sulphur dioxide solution. Place a few glass beads in the beaker, and evaporate the solution, with vigorous boiling, to 60 ± 5 ml. Dilute to 300 ml with water, and heat the solution to a temperature between 80° and 90° C. Set the polarising voltage to 0·2 volts, and adjust the electrodes so that they are just below the surface of the solution. Titrate with 0·1 N potassium bromate until the meter shows a permanent deflection.

RESULTS—

This method has been in use in our laboratory for several years for determining antimony in antimony - lead alloys with an antimony content from 10 to 90 per cent. and in tin - lead solders with an antimony content between 0·2 and 5 per cent. The results obtained by this method agree with those obtained by using methyl orange, and the considerable number of results exchanged with other laboratories on these types of materials have been in good agreement. To check the reproducibility of the method an antimony - lead alloy was analysed by several different operators. An average of 11·23 per cent. of antimony was obtained with a standard deviation of 0·05 per cent.

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A Rapid Solvent-extraction Sampling Technique for Neutron-activation Analysis

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A solvent-extraction sampling technique has been used for comparing the specific activity of an element separated from an irradiated sample with that of an irradiated standard after both have been diluted with inactive carrier. The method has been applied to provide a rapid separation and determination of silver, the activity of the 2·3-minute silver-108 nuclide being measured.

PRECIPITATION techniques have been widely applied in neutron-activation analysis, as a means of assessing chemical yield, when separation is required to obtain the element to be determined in a radiochemically pure form.

When the activity of short-lived nuclides is to be measured, however, the time needed for precipitation is often inconveniently long, and other methods, for example, spectrophotometry after liquid counting,[1,2] have been employed for determining the yield. These methods are usually designed to assess the total yield of elements, thus permitting the specific activity to be calculated after the radioactivity has been assayed. The amount of element in the original sample can then be found by comparing this specific activity with that of an irradiated standard, inactive carrier having been added to both sample and standard at the start of processing.

An alternative procedure would be to measure the radioactivity of the same amount, rather than total yield, of element derived from sample and from standard, after carrier has been added; simple comparison of activities will then give the ratio of the specific activities in the two cases. Although some decrease in sensitivity will result from measuring only part of the final yield, this might well be off-set by the expediency of the method when measurement of radioactivity soon after completion of irradiation is essential.

Recently, a solvent-extraction sampling method has been proposed for a tracer dilution technique[3] in which a solution of constant metal concentration is prepared by virtually complete conversion of a known amount of complexing agent, HL, to the corresponding complex, ML_n, with the metal to be determined M^{n+}, ML_n being extracted into a known volume of organic phase. This type of sampling should be applicable to activation analysis, and further, since HL will form a complex with M^{n+} in preference to other metals present in the system giving weaker complexes than ML_n, the solvent-extraction step will impose some restriction on the number of elements likely to interfere. We report here investigations carried out to assess the possibility of applying solvent-extraction sampling to neutron-activation procedures with particular reference to a method devised for the rapid determination of silver in which the activity of the short-lived 2·3-minute silver-108 nuclide is measured. A solution of diphenylthiocarbazone (dithizone) in carbon tetrachloride was used to extract the silver.

EXPERIMENTAL

Commercially available dithizone (Hopkin and Williams Ltd.) was dissolved in carbon tetrachloride, extracted into dilute aqueous ammonia solution, and the aqueous phase washed with fresh organic solvent. After the solution had been acidified with hydrochloric acid, the precipitated dithizone was dissolved in carbon tetrachloride and its purity assessed by measurement of the ratios of the optical densities at the absorption maxima at 620 and 450 mμ and the minimum at 510 mμ. Purification by extraction was repeated, if necessary, until satisfactory values were obtained. The concentration of the dithizone solutions was calculated from the absorption at 620 mμ of a sample (diluted if necessary), assuming a molecular extinction coefficient[4] of $34{\cdot}6 \times 10^3$.

All glassware was made of Pyrex glass, and for experiments in which tracer was required 253-day silver-110*m* was used.

Irradiations necessary for activation analyses were carried out by sealing samples and standards in thin-wall 6-mm silica tubes and irradiating in a flux of approximately

10^{12} neutrons per sq. cm per second, a pneumatic-transfer system being used. "Doped" samples of known silver content were prepared by weighing out about 0·1 g of iron or steel found to have no detectable silver content into 6-mm silica tubes, and adding known amounts of silver in a few microlitres of solution. The silica tubes were then sealed and irradiated in the usual way.

Chemical techniques and gamma liquid-counting methods involving use of a well-type scintillation counter were perfectly standard and will not be mentioned further; an M6H type liquid beta counter was used for beta counting. Kemp and Smales[1] have described a convenient method for liquid counting the beta activity of sample and standard alternately.

The activation analysis procedure is described and discussed in the section dealing with the determination of silver, p. 606.

RESULTS AND DISCUSSION

GENERAL CONSIDERATIONS—

The solvent-extraction sampling technique may be most conveniently applied when reagent HL is virtually completely converted to ML_n in the presence of excess of M^{n+} over a wide range of conditions. Dithizone was therefore chosen as extractant for silver, since the reagent is known to form a strong primary silver complex in acid solution, which is soluble in carbon tetrachloride, but nearly insoluble in water.[5] The reliable stoicheiometry of the 1 + 1 silver - dithizone complex formed in acid solution, also suggests that it should be possible to prepare a standard silver dithizonate solution from a solution of dithizone in carbon tetrachloride of known concentration in the presence of excess of silver under suitable conditions.

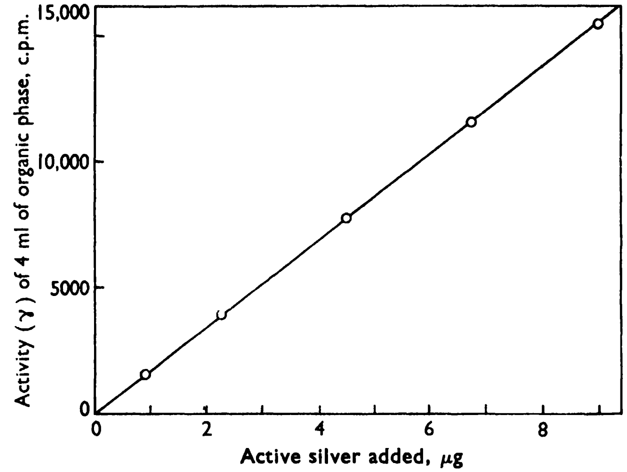

Fig. 1. Dependency of count rate of silver dithizonate phase (c_x) on amount of active silver added

If the specific activity, S_1, of an element under standard counting conditions is given by $S_1 = c_1/m_1$ counts per minute per g, where c_1 is the absolute count rate in counts per minute of m_1 grams of material, then when m_1 grams of the active element are added to M grams of inactive carrier, after equilibration the new specific activity S_2 will be given by $S_2 = c_1/(m_1 + M)$ counts per minute per g.

Thus the count rate c_x of X grams of diluted element will be—

$$c_x = \frac{c_1}{(m_1 + M)} . X = \frac{S_1 X}{(m_1 + M)} m_1 \qquad \text{.. .. ..} \qquad (1)$$

When activation analysis is used for determining trace elements, the amount of active element isolated from a sample (m_1) is likely to be small compared with carrier (M), and therefore, if X, S_1 and M are kept constant, there should be a linear dependency of c_x upon m_1. This was confirmed by shaking 5 ml of a solution of dithizone in carbon tetrachloride with a 0·5 M sulphuric acid phase, containing small amounts of active silver and 750 μg of inactive silver carrier, there being enough dithizone present to convert only 375 μg of silver into the complex. The count of 4 ml of the organic phase as a function of the active silver added is shown in Fig. 1, from which it can be seen that the predicted linear relationship is found.

INTERFERENCES—

Since solvent-extraction sampling assumes complete conversion of reagent HL to complex ML_n, extraction of elements, other than the one to be determined, will result in the measurement of the activity of smaller amounts of M^{n+} than theoretically expected, thereby introducing error. Further, when solvent-extraction sampling is being used in activation analysis procedures, although the amounts of elements extracted together with ML_n may not be large enough to affect, appreciably, the amount of M^{n+} whose activity is measured, there still may be sufficient impurity present in the organic phase to interfere seriously with the radiochemical purity of the solution of ML_n to be measured.

If reaction between dithizone (here represented as the monobasic acid HL) and two metals M_1^{n+} and M_2^{p+}, is given by—

$$M_1^{n+} + nHL \rightleftharpoons M_1L_n + nH^+ \qquad (2)$$

and

$$M_2^{p+} + pHL \rightleftharpoons M_2L_p + pH^+ \qquad (3)$$

then

$$\frac{[M_1\,L_n]_o}{[M_2\,L_p]_o} = \frac{K_1\,[M_1^{n+}]}{K_2\,[M_2^{p+}]}\,\frac{[H^+]^{p-n}}{[HL]_o^{p-n}} \qquad (4)$$

where K_1 and K_2 are equilibrium constants for equations (2) and (3), and the subscript, $_o$, refers to species present in the organic phase. Elements likely to interfere when solutions of dithizone are used for solvent-extraction sampling have already been considered with particular reference to the tracer dilution technique[3] and therefore will not be re-considered here. It can be seen from equation (4), however, that a high $[M_1L_n]_o/[M_2L_p]_o$ value is favoured by a larger ratio of equilibrium constant K_1/K_2, and interference to the extraction of silver would be expected from mercury, palladium and gold—elements known to form extremely stable dithizonates.[5]

To assess interference with the extraction of silver caused by the presence of other elements, approximately N sulphuric acid phases were prepared containing 750 μg of inactive silver, 4·5 μg of active silver tracer and known amounts of other inactive elements. Aqueous phases were shaken with 5 ml of a solution of dithizone, containing sufficient reagent to extract a total of 375 μg of silver, and the radioactivity of 5 ml of the organic phase was counted. Results shown in Table I indicate that mercury, palladium and gold do interfere, but the presence of large amounts of other elements known to form well defined dithizonates does not decrease the count from the silver found in the organic phase.

TABLE I

INTERFERENCE WITH THE EXTRACTION OF SILVER CAUSED BY THE ADDITION OF INACTIVE ELEMENTS

One milligram of each element was added

Element added	Count rate of 4 ml of organic phase, counts per minute	Element added	Count rate of 4 ml of organic phase, counts per minute
Silver only present	15,366		
Manganese	15,229	Tin^{II}	15,168
Iron	15,292	Lead	14,912
Cobalt	15,069	Copper	15,520
Nickel	15,143	Bismuth	15,350
Zinc	15,175	Gold	64
Cadmium	15,280	Mercury	1
Indium	15,119	Palladium	407

Separation of interfering elements from the silver could be carried out by conventional chemical separation procedures. As, however, it was hoped to devise a neutron-activation method based on the measurement of the short-lived 2·3-minute silver-108 nuclide, the time available for any processing was severely limited.

Mercury, palladium and gold may be removed by a preliminary extraction with dithizone. If the expected total amount of these metals is small compared with the silver carrier added, the dithizone necessary for their extraction will cause only a small loss of silver from the system, even in the extreme instance when the interfering metals are not present. Any decrease in sensitivity owing to the extraction of some silver at this stage could well be off-set by the short time needed to complete the separation, which is likely to be less than that required to separate the 2·3-minute nuclide by conventional means. A series of experiments was carried out, similar to those described above, which provided the results in Table I, except that the aqueous phase was first shaken with 10 ml of 10^{-4} M dithizone in carbon tetrachloride. Under these conditions it was found that the amounts of these elements added (up to a total of 30 μg) caused no decrease in the amount of silver finally extracted.

USE OF SOLVENT-EXTRACTION SAMPLING FOR DETERMINING SILVER BY NEUTRON ACTIVATION

In order to investigate the possibility of determining silver by assay of the radioactivity of the 2·3-minute silver-108 nuclide, the silver contents of a number of "doped" samples were measured. After irradiation, the silica tube containing the sample was opened and dropped, with its contents, into 10 ml of diluted nitric acid (1 + 1) containing 1000 μg of silver carrier. When the sample had dissolved, the resulting acid solution was diluted to about 20 ml with distilled water, and hydroxides were precipitated by addition of ammonia solution to provide a preliminary separation of many elements from silver. After heat had been applied for a few seconds, the precipitate was removed on a Whatman No. 541 filter-paper, and the filtrate was collected in a beaker cooled by ice, made approximately 0·5 N in sulphuric acid, and 2 ml of 20 per cent. w/v hydroxylamine sulphate were added. The acid silver solution was then transferred to a separating funnel containing 10 ml of 10^{-4} M dithizone in carbon tetrachloride, the funnel and its contents were shaken for about 20 seconds (longer if palladium was present), and the phases were separated by spinning in a centrifuge. This step not only removed interfering elements but also pre-saturated the aqueous phase with organic solvent. The aqueous phase was transferred to a second separating funnel containing 5 ml of $7{\cdot}4 \times 10^{-4}$ M dithizone in carbon tetrachloride, experiments with inactive silver and active tracer having shown that a yield of at least 50 per cent. could be expected at this stage.

The funnel and contents were shaken for about 20 seconds, the phases separated by centrifugation, and 4 ml of the organic phase was placed by pipette in a polythene container.

When convenient, the silver standard was added to 1000 μg of inactive carrier in 0·5 N sulphuric acid, warmed to achieve chemical equilibrium, cooled, and 2 ml of 20 per cent. w/v hydroxylamine sulphate solution were added. The aqueous phase was extracted with two dithizone solutions in the same way as described above in the procedure for the sample, and 4 ml of silver dithizonate solution were again transferred by pipette to a polythene container. Sample and standard were counted alternately to assess the radioactivity of the solutions, and the decay curves of the nuclides were plotted.

In some experiments it was found that the silver was contaminated with activity from small amounts of copper. Although silver will replace copper from dithizone solution, and in fact copper dithizonate may be used as an absorptiometric reagent for silver, it is clear from equation (4) that some copper must be extracted with the silver into the dithizone phase. Usually, the amount is small and has a negligible effect (*e.g.*, see Table I), but the great sensitivity of many elements to neutron activation may result in minute amounts of extracted elements leading to appreciable radiochemical interference.

The contribution of copper to the radioactivity of the sample could be found by resolution of the decay curve obtained, but ethylenediaminetetra-acetic acid (EDTA) has been used as masking agent for small amounts of copper when silver is being extracted by a solution of dithizone.[6] Consequently, when copper was present in the sample, EDTA was added to the final aqueous phase, the conditions used being similar to those already proposed by Friedeberg.[6]

For a number of samples beta- rather than gamma-counting was used, since the higher sensitivity to silver activity and lower background of beta-counting were preferred to the greater convenience of gamma-counting. For beta-counting 2000 μg of silver carrier were added to sample and standard, 15 ml of dithizone solution were used for the final extraction, instead of 5 ml, and the activity of 10 ml was counted.

From equation (1) it can be seen that the count rates c_{x1} and c_{x2} of silver dithizonate derived from m_1 grams of silver standard and m_2 grams of carrier, will be—

$$c_{x1} = \frac{S_1 X}{(m_1 + M)} m_1 \text{ and } c_{x2} = \frac{S_1 X}{(m_2 + M)} m_2.$$

The specific activity, S_1, will be the same for both if sample and standard are irradiated in a similar neutron flux, and no self-shielding occurs; thus if M is large compared with m_1 and m_2, then—

$$c_{x1}/c_{x2} = m_1/m_2.$$

Results obtained for the analysis of several "doped" samples by measuring the 2·3-minute activity of the silver-108 nuclide are shown in Table II, together with the type of counter used.

Table II

Analysis of "doped" samples for silver by measurement of the activity of the 2·3-minute ^{108}Ag nuclide

Inactive silver added, µg	Silver content calculated, p.p.m.	Silver content, found, p.p.m.	Counter used
12·0	102·5	108·0	γ
7·9	75·2	75·0	γ
5·2	42·7	36·3	γ
1·06	7·07	9·84	γ
0·74	4·95	5·72	γ
3·77	36·0	34·2	β
1·25	10·3	10·5	β
1·24	7·6	7·08	β
0·38	3·42	3·21	β

The time elapsing between receipt of the sample in the laboratory and the start of counting was usually about 8½ minutes; a further 2 minutes were required for transferring the sample and standard from reactor to laboratory, so that counting usually began about 5 half-lives after the completion of irradiation. Under these conditions and with the counting techniques used, the lower limits of determination given by count rates of 600 gamma-counts per minute and 200 beta-counts per minute above background at the start of counting were found to be ~0·4 µg and ~0·06 µg, respectively. It should, however, be noted that the samples could be completely dissolved in less than 1 minute, and a longer time is likely to delay the start of counting, thus reducing the sensitivity of the method.

The sensitivity achieved by measuring the silver-108 activity 10 minutes after the completion of irradiation is not as high as that estimated for measurement of the activity of the 253-day silver-110*m* nuclide (5×10^{-9} g after 1 month's irradiation[7]). However, for the determination of more than 10^{-7} g of silver, the short irradiation time and rapid completion of analysis when the shorter-lived nuclide is used for measurement does present advantages.

(Since this paper was submitted for publication an article[8] has appeared in which are considered some theoretical aspects of the solvent-extraction sampling technique as applied to activation analysis.)

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Application of the AutoAnalyzer to the Determination of Zinc in Soils and Sediments

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The AutoAnalyzer is adapted to the determination of zinc with dithizone. A solvent-extraction technique, which requires only simple modifications of the system, is applied to the analysis of solutions from soil and sediment samples containing as little as 0·1 µg of zinc per ml. Samples are analysed at the rate of 250 per day, and at this speed the results obtained are within ±5 to 10 per cent. at the 95 per cent. confidence level.

A FIELD method for determining zinc in soils, suitable for geochemical prospecting, has been developed by Lakin, Stevens and Almond.[1] The procedure[2] in current use at the Geochemical Prospecting Research Centre, Imperial College, was derived from this method; it consists of the visual comparison of sample solutions with a standard series and makes use of the mixed colour reaction of zinc with dithizone in benzene solution. Concentrations over the range 5 to 350 p.p.m. are determined from a portion representing 10 mg of the sample. This is typical of many methods now available for geochemical field work, and permits the analysis of 100 samples by one person in an 8-hour day. That this rate of analysis can be achieved is due largely to the fact that results within ±25 per cent. at the 95 per cent. confidence level are adequate for most prospecting purposes.

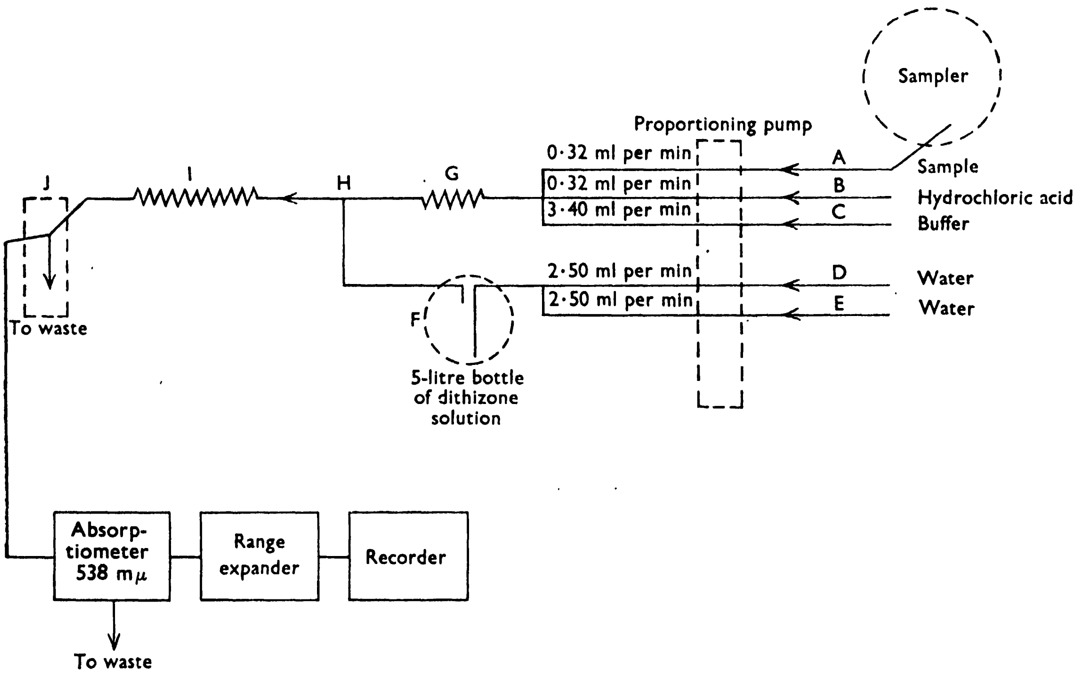

Fig. 1. Arrangement of apparatus

The Technicon AutoAnalyzer was conceived by Skeggs,[3] and the mechanics and principles have been discussed by Ferrari, Russo-Alesi and Kelly.[4] The geochemical field method for zinc has been adapted to this system, resulting in an improvement in both analytical precision and the lower limit of determination, together with an increased speed of analysis.

DESCRIPTION OF APPARATUS

Proportioning pump—The manifold assembly is shown diagrammatically in Fig. 1, the pumping action being such that solutions progress from right to left.

Sampler—The Technicon "Super-Sampler" consists of a circular aluminium rack that holds test-tubes (19 mm × 150 mm) in four concentric circular paths of 50 tubes each. With the pump in action, a solenoid-operated polythene sampling tube drops vertically into a test-tube and aspirates sample solution for 1 minute (Fig. 1, tube A); at the end of this period the tube rises to aspirate air, and on completion of a total cycle time of 2 minutes the rack rotates sufficiently to present the next sample, and the tube falls again. A modification has been made to the arm supporting the sampling tube so that when this tube rises to aspirate air, a second tube, identical in bore and length, falls into 0·5 N hydrochloric acid (Fig. 1, tube B). The alternate intake of sample solution and acid ensures a constant volume of air and aqueous phase, which is essential for the subsequent phase-separation stage.

The total cycle and sampling times are pre-set by means of two independent controls, each of which extends to 35 minutes with sub-divisions of 30 seconds. However, it is mechanically impossible to operate with a sample time shorter than 1 minute without modification of the equipment.

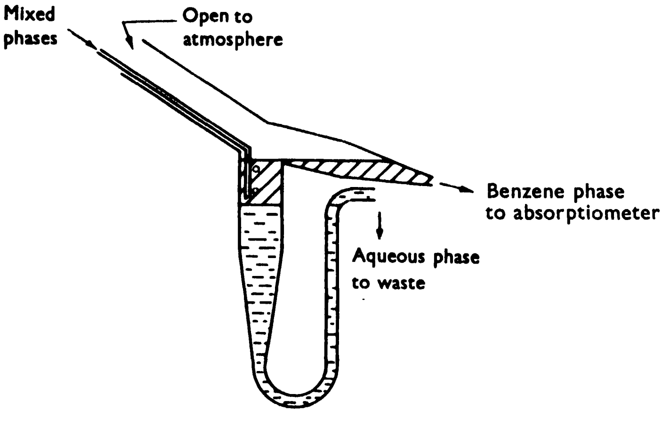

Fig. 2. Modified Y-tube

Solvent extraction—Since the Tygon pump tubing is not resistant to benzene, dithizone dissolved in this solvent is introduced into the system by displacement with water, which is pumped through tubes D and E (Fig. 1) into a 5-litre darkened bottle (at F) containing the dithizone. At G, the aqueous solutions and air (tubes A, B and C) pass through a coil of glass tubing of 2-mm bore with 28 turns approximately 1·5 cm in external diameter, in order to mix sufficiently before meeting the dithizone stream at H. This junction is made with a glass h-piece containing a constriction, which has the effect of distributing the benzene phase as a series of small globules throughout the aqueous solution, and the combined streams then circulate through seven more mixing coils at I. The mixing coil assembly lies horizontally, and zinc dithizonate is extracted into the benzene phase by repeated inversion of the mixed phases as they move along. An adequate extraction is achieved by the time the stream enters (at J) the modified Y-tube (see Fig. 2), where the phases separate and the benzene solution passes into the flow cuvette of the absorptiometer, the aqueous phase running to waste.

Absorptiometer—A flow cell of 1-cm light path is used with 538-mμ filters. After passage through the cell, the solution runs to waste through a tube at the bottom of the absorptiometer. The drainage tube supplied with the instrument is made of material unsuitable for use with benzene, and it must be replaced; a glass condenser adapter is a convenient substitute.

Range expander—The photocell response is received by this unit, and it may be magnified by a factor of 2, 4 or 10 before transmission to the recorder.

Recorder—This is operated with a chart speed of 10 inches per hour.

METHOD

REAGENTS—

Potassium hydrogen sulphate, fused, powder.

Hydrochloric acid, N *and* 0·5 N—Prepared from concentrated acid of analytical-reagent grade.

Dithizone solution—A 0·01 per cent. w/v solution in carbon tetrachloride, and a 0·001 per cent. w/v solution in benzene prepared each day from a 0·02 per cent. w/v stock solution in benzene. Use analytical-reagent grade dithizone for preparing these solutions.

Carbon tetrachloride—Analytical-reagent grade.

Benzene—Crystallisable grade.

Acetate buffer solution—Dissolve 500 g of sodium acetate trihydrate, 125 g of sodium thiosulphate pentahydrate and 5 g of sodium fluoride in about 1·5 litres of water, add 15 ml of glacial acetic acid, and extract with 0·01 per cent. dithizone in carbon tetrachloride until free from zinc. Remove the excess of dithizone by extraction with carbon tetrachloride, and then dilute to 2 litres with water.

Standard zinc solutions—Dissolve 0·2199 g of analytical-reagent grade zinc sulphate heptahydrate in 0·5 N hydrochloric acid, and dilute with the same acid to 500 ml in a calibrated flask. From this solution (containing 100 μg of zinc per ml) prepare as required, by dilution with 0·5 N hydrochloric acid, solutions containing from 0·1 to 10 μg of zinc per ml.

PROCEDURE—

Weigh 0·1 g of the sifted sample, place in a numbered 19-mm × 150-mm borosilicate-glass test-tube, mix with 0·5 g of potassium hydrogen sulphate powder, and fuse. Cool, leach the residue with 5 ml of N hydrochloric acid, dilute to 10 ml with water, and mix.

Assemble the manifold as indicated in Fig. 1, and connect to the appropriate reagent solutions. Insert 538-mμ filters and a 1-cm flow cell, set the range expander at 1, bring the pump into operation, and allow the supplementary sample-line to aspirate 0·5 N hydrochloric acid continuously. Adjust the recorder and absorptiometer to read zero transmission when no light is passing through the flow-cell; then select a convenient magnification factor on the range expander, and obtain an arbitrary base-level for 100 per cent. transmission.

Load the sampler rack with sample solutions, several solutions for the determination of reagent blank values, standard solutions at intervals of 1 in 20, control samples at a frequency of 1 in 40, and test-tubes containing only 0·5 N hydrochloric acid at an incidence of 1 in 40. Set the sampler unit in operation with a total cycle of 2 minutes and a sampling time of 1 minute.

After the last solution has been sampled, allow the supplementary line to aspirate 0·5 N hydrochloric acid for a further 15 minutes, until the final peak has been traced. Switch off the system, isolate the bottle containing the solution of dithizone in benzene, place the reagent lines in water, pump for a further 5 minutes, and then release the roller-head from the platen of the proportioning pump. Remove the recorder chart, and draw a baseline through all the zero levels obtained from the 0·5 N acid solutions and the initial setting. Measure the optical density directly from this line by using a ruler calibrated with the appropriate logarithmic scale. Make a graphical plot of the results obtained for standard solutions and interpolate the zinc concentrations of the sample solutions. When the zinc concentration is outside the range covered by the magnification factor selected on the range expander, adjust this unit accordingly and repeat the determination.

DISCUSSION OF RESULTS

For prospecting purposes, it is only necessary to take into account cobalt, copper, lead and nickel when considering the effect on the determination of zinc of other metals that react with dithizone. The aqueous phase will have a pH value in the range 5·6 to 6·0, within which there is no appreciable reaction by nickel. The reactions of the other three metals are not completely prevented by sodium thiosulphate, and their dithizonates have absorption maxima too close to that of zinc dithizonate for the optical filters to discriminate; from solutions each containing 100 μg of cobalt, copper and lead per ml, apparent zinc values of approximately 10, 3 and 1 μg per ml will be obtained, respectively. These degrees of interference are tolerable for the proposed application.

Fluoride is necessary in the buffer solution to mask aluminium, which would otherwise inhibit either the formation or the extraction of zinc dithizonate (or both) when present at a concentration greater than 150 times that of zinc.

Slight decomposition of the dithizone solution may occur, causing a drift of the baseline. With a magnification factor of 4 or 10, the fall in base-level should not exceed 0·02 or 0·04 optical-density units, respectively, over an 8-hour period; this is equivalent to about 0·05 μg of zinc per ml in each instance. When there is a greater change in the baseline, either the dithizone is too unstable to be used or else the drift is indicative of some instrumental fault.

Alternative means of decomposing the sample are not precluded, such as digestion with perchloric acid or aqua regia, or the preliminary removal of silica by treatment with hydrofluoric acid. The sample attack is merely restricted by its subsequent chemical effect and the need to obtain a final aqueous phase with the same pH value and concentrations of fluoride and thiosulphate as those obtained in the procedure described here. It is convenient to adopt a method of attack that can be carried out in test-tubes, since these are necessary to contain the solutions in the sampler unit.

Sample solutions contain an appreciable amount of suspended solid matter (mainly siliceous) that is carried into the system with the sample stream and collects at the aqueous - benzene interface in the phase-separation cell. In early experiments, air was introduced continuously to promote mixing of the benzene and aqueous layers, but when escaping at the phase-separation stage it caused some solids to be carried over into the flow cell of the absorptiometer, which gave rise to erratic tracing on the recorder chart; the air intake was minimised accordingly.

Optimum conditions for solvent extraction were established experimentally with up to twelve mixing coils. Centrifugation occurred with eight or more coils, causing coalescence of the previously segmented benzene stream and thereby spasmodic overflow into the absorptiometer cell, which resulted in a chart tracing of poor quality. Zinc dithizonate is extracted less rapidly from sample than from standard solutions, and care was taken to ensure that the same degree of extraction from both was achieved.

It is essential to calibrate the pump tubes before assembling the manifold. Mostly, they are within ±15 per cent. of their nominal value, but it has been found possible to deviate seriously from the optimum proportions of sample and reagent solutions by taking the tubes at their face value.

Some figures for precision are shown in Table I. Results on control samples distributed among 550 routine samples have been used for calculating standard deviations; the same solutions were also analysed successively at as many amplification factors as possible.

A comparison of results obtained manually and by the AutoAnalyzer is shown in Table II.

TABLE I

REPRODUCIBILITY OF RESULTS FOR ZINC

Sample	No. of determinations	Mean value, μg per ml	Standard deviation	Range expander setting
From routine operation –				
A	6	0·28	0·019	10
A	8	0·30	0·021	4
B	6	0·12	0·011	10
C	6	1·23	0·053	4
C	5	1·16	0·023	2
D	6	3·01	0·13	2
From consecutive analyses – –				
A	5	0·27	0·009	10
A	7	0·29	0·006	4
B	7	0·11	0·003	10
C	7	1·18	0·054	4
C	7	1·13	0·050	2
C	7	1·28	0·025	1
D	7	3·00	0·092	2
D	7	3·00	0·105	1

The results in Table II were obtained on a set of control samples prepared according to Craven's recommendations.[5] Two bulk samples were obtained, one (*h*) containing about 380 p.p.m. of zinc and the other (*l*) about 35 p.p.m. of zinc; a series of twelve samples was

TABLE II

COMPARISON OF RESULTS OBTAINED MANUALLY AND WITH THE AUTOANALYZER

Sample No.	Zinc found— manually, p.p.m.	automatically, p.p.m.	Sample No.	Zinc found— manually, p.p.m.	automatically, p.p.m.
1	200	213	7	140	125
2	400	385	8	40	40
3	250	268	9	270	308
4	200	163	10	30	43
5	380	363	11	50	55
6	190	208	12	210	213

prepared by mixing different amounts of the two bulk samples. For each sample in this series, the weight fraction of the high component is designated θ and that of the low component λ, so that $\theta = h/(h + l)$ and $\lambda = l/(h + l)$ for any given sample. When the twelve samples had been analysed, values for the high and low components (H and L, respectively) were calculated from—

$$H = \frac{\Sigma \mathrm{A}\theta \Sigma \lambda^2 - \Sigma \mathrm{A}\lambda \Sigma \theta\lambda}{\Sigma \theta^2 \Sigma \lambda^2 - (\Sigma \theta\lambda)^2}$$

and $$L = \frac{\Sigma \mathrm{A}\lambda \Sigma \theta^2 - \Sigma \mathrm{A}\theta \Sigma \theta\lambda}{\Sigma \theta^2 \Sigma \lambda^2 - (\Sigma \theta\lambda)^2}$$

where A is the analytical result found for a sample. A value was then calculated for each sample, from $\hat{\mathrm{A}} = H\theta + L\lambda$, and the standard deviation was obtained from—

$$\sigma = \sqrt{\Sigma\,(\mathrm{A} - \hat{\mathrm{A}})^2/(\mathrm{n} - 2)}.$$

By Craven's definition the percentage mean accuracy is obtained from $(100 \times 2\sigma)/\frac{1}{2}(H + L)$. The results in Table II when treated in this manner give—

	H	L	Mean accuracy, %
Manual	383	34	22·7
Automatic	385	36	9·0

From this statistical treatment it may be presumed that there is good agreement between the two methods, but that the results from automatic analysis are more mutually consistent than are those obtained manually.

TABLE III

CONCENTRATION RANGE FOR EACH RANGE EXPANDER SETTING

Expansion factor	Range of zinc concentrations, μg per ml Linear	Non-linear
1	0·25 to 4·00	4·0 to 10·0
2	0·10 to 2·00	2·0 to 4·0
4	0·05 to 1·00	1·0 to 2·0
10	0·01 to 0·50	0·50 to 0·75

The calibration graph for each setting of the range expander is not linear over its full extent; the ranges are shown in Table III. Interpolation from the curved section of the calibration graph has proved to be satisfactory, but for a large number of samples it is likely to be quicker to repeat at a lower sensitivity.

By using a sample solution, the effect of variations in the timing of the sampler unit operation was studied to discover how much the precision and the lower limit of the determination might be improved at the expense of the speed of analysis (see Table IV). By increasing the sampling time to 2 minutes, the sensitivity of the determination is improved by 15 per cent., but a further improvement of only 4 per cent. is obtained with a sampling period of 3 minutes.

The start-up procedure and the final flushing out of the system may be accelerated by use of a two-speed proportioning pump. This can operate at a fast pumping rate for 3 or 4 minutes and gives an additional 30 to 40 minutes of analytical time. Without this advantage, the equipment has been left unattended for 2 or 3 hours at a time and allowed to run during the lunch-hour, and one person can thus analyse 250 samples in an 8-hour working day, in addition to control samples and standard solutions. One sample takes about 15 minutes to be processed by the system.

TABLE IV

REPRODUCIBILITY AT DIFFERENT TOTAL CYCLE AND SAMPLING TIMES

Total cycle, minutes	Sampling time, minutes	No. of determinations	Zinc concentration Mean, μg per ml	Zinc concentration Standard deviation
2	1	19	1·97	0·038
3	1	10	1·99	0·032
4	1	9	2·02	0·026
3	2	10	2·00	0·049
4	2	10	2·02	0·045
5	3	10	2·01	0·028

Although no sample solutions with less than 0·1 μg of zinc per ml were analysed, a lower limit of 0·01 μg per ml can be attained. When this is necessary, it would be advisable to use a total cycle of 5 minutes with a sampling time of 3 minutes.

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The Polarographic Determination of Copper, Cadmium, Thallium, Lead, Tellurium and Iron in Selenium

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A polarographic method has been developed for determining copper, cadmium, thallium, lead, tellurium and iron in high purity selenium used for making rectifiers. The selenium matrix is removed completely by distillation as selenium bromide from hydrobromic acid solution. The residue is dissolved in specially purified oxalic acid solution, which acts as the electrolyte.

On a 2-g sample of selenium, 0·2 p.p.m. of copper, 0·02 p.p.m. of lead, 0·0015 p.p.m. of tellurium, 0·01 p.p.m. of cadmium, 0·05 p.p.m. of thallium and 0·4 p.p.m. of iron can be determined in 1 ml of solution. The limits of detection for copper and iron are poor owing to relatively high blank values of 0·1 μg and 0·2 μg, respectively.

ELEMENTAL selenium is used for making rectifiers, the electrical characteristics of which are affected by the presence of metallic impurities. Relatively little has been published on the determination of trace impurities in selenium.[1,2,3,4]

Peterson and Currier[1] spectrographically determined 1 to 100 p.p.m. of aluminium, bismuth, copper, lead, magnesium, nickel, silver and zinc in selenium.

Itsuki and Kaji[2] determined copper and lead in high purity selenium with a square wave polarograph. They employed perchloric acid as electrolyte, after removing the selenium with sulphuric and nitric acids.

Pohl[3] determined thallium, iron, copper, cadmium, bismuth and lead polarographically in selenium. He dissolved the selenium in nitric acid, removed the selenium dioxide by volatilisation in a muffle furnace, and separated the iron and thallium by extraction with di-isopropyl ether from 6 N hydrochloric acid. After the solution had been evaporated to dryness and the residues dissolved in ammonium tartrate, the other metals could be determined.

Jones[4] determined tellurium, lead, copper, nickel and cobalt polarographically in selenium. He removed the selenium dioxide by volatilisation from hot concentrated sulphuric acid solution. The tellurium was separated from the other elements by precipitation with sulphur dioxide. The copper and lead were determined in 0·1 N hydrochloric acid - 0·1 N potassium chloride solution after reduction of the iron to the ferrous state with hydroxylamine hydrochloride. The cobalt and nickel were determined in pyridine solution, and the tellurium determined in ammonium hydroxide - ammonium chloride electrolyte.

An advantage of the polarographic method is that more than one element can readily be determined on the same prepared sample solution. However, selenium in most electrolytes produces large reduction and catalytic waves, which would seriously interfere with the determination of many other elements. It is therefore necessary to remove selenium before making the final determination.

Selenium may be removed by precipitation, but many other elements are co-precipitated,[5] and so this method was not used. Distillation from concentrated sulphuric acid solution has been reported,[4] but it is necessary to purify the sulphuric acid before use to limit the blank values for the whole procedure. A more convenient process involves distillation as the bromide from hydrobromic acid solution.[6] With this technique most elements remain as involatile bromides.

Briefly, the method described here involves dissolution of the sample in a mixture of nitric and hydrochloric acids, removal of the excess of nitric acid, distillation of selenium bromide, and finally dissolution of the residue in the appropriate electrolyte, which is examined polarographically. A simple but extremely effective heater assembly is used for evaporating liquids in beakers, which has a provision for minimising contamination by airborne impurities (see Fig. 1).

METHOD

REAGENTS—

Hydrochloric acid, 6 *to* 7 N—Distil 7 N hydrochloric acid in quartz apparatus.

Nitric acid—Distil analytical-reagent grade concentrated nitric acid in quartz apparatus.

Hydrobromic acid—Distil 48 per cent. hydrobromic acid, of analytical-reagent grade, in quartz apparatus.

Oxalic acid, 0·2 M—Dissolve 25 g of analytical-reagent grade oxalic acid in 1 litre of water, and pass the solution through an ion-exchange column packed with analytical-grade Amberlite IR-120(H).

2 M *Ammonium acetate* - 2 M *acetic acid* - 0·004 M *EDTA solution*—Prepare a solution of 1·5 g of analytical-reagent grade disodium ethylenediaminetetra-acetate in 230 ml of redistilled analytical-reagent grade acetic acid and 145 ml of redistilled analytical-reagent grade ammonia solution. Dilute to 1 litre with water distilled in quartz apparatus.

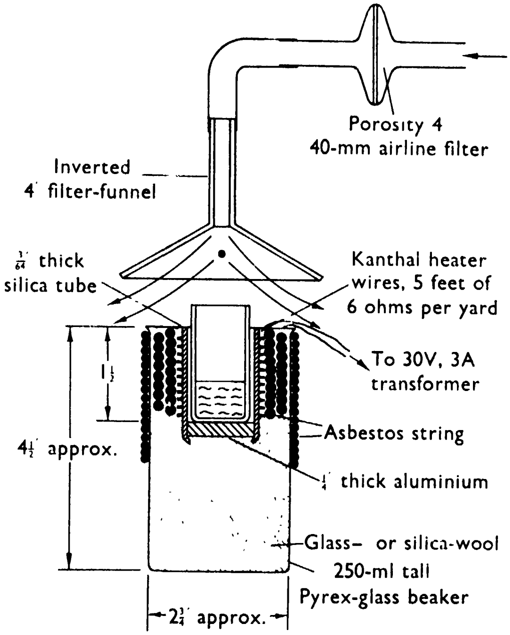

Fig. 1. 50-ml beaker heater with down-draught assembly

SEPARATION PROCEDURE—

Place 2 g of sample in a 50-ml quartz beaker, and dissolve in 5 ml of (1 + 1) hydrochloric acid and 10 ml of concentrated nitric acid. When the reaction has subsided place the beaker in the heater assembly provided with a down-draught of filtered air (see Fig. 1), and evaporate the solution to dryness. After allowing the residue to cool slightly, carefully add 20 ml of hydrobromic acid, replace the mixture in the heater assembly, and evaporate off the selenium as the tetrabromide. It is important at this stage that the temperature of the heater is not above 155° C (measured with a thermocouple between the beaker and heater wall) otherwise some tellurium will be lost as tellurium bromide, boiling-point 421° C.

After the solution has been evaporated to dryness and the residue cooled slightly, add a further 1 ml of nitric acid to re-convert into selenious acid any elemental selenium that may have been formed. Transfer the solution obtained to a 10-ml calibrated flask, and make up to the mark with distilled water. Transfer two separate 4-ml portions to 50-ml quartz beakers, and evaporate to dryness as before. Add to the contents of each beaker 1 ml of hydrobromic acid, and remove the remaining selenium as described above. The residues thus obtained are polarographically free from selenium and contain the trace-impurity metals as bromides.

POLAROGRAPHIC PROCEDURE—

A Southern Instruments' A1660 Davis differential cathode-ray polarograph was used. The approximate reduction potentials of the elements in oxalic acid at pH 2 are—

Element	Iron^{III}	Copper	Titanium	Lead	Thallium	Cadmium	Tellurium
Reduction potential, volts	0·15	0·25	0·60	0·65	0·65	0·75	0·95

DETERMINATION OF IRON, COPPER, CADMIUM, TELLURIUM AND COMBINED LEAD AND THALLIUM—

Dissolve the residue from one of the beakers (see "Separation Procedure") in 2 drops of 0·1 N hydrochloric acid and then 0·5 ml of 0·2 M oxalic acid. Transfer the solution to a 1-ml calibrated flask by means of a glass dropping tube. Rinse the beaker with water, and transfer the rinsings to the 1-ml flask. Add 1 drop of 0·5 N ammonium hydroxide to the solution in the flask, in order to neutralise the hydrochloric acid. Make the solution up to the mark with distilled water, and transfer the solution to a semi-micro polarographic cell. Prepare a reagent blank solution treated in a similar manner to the sample, and place it in the other cell of the differential polarograph.

De-oxygenate the oxalic acid solutions in the polarographic cells with pure nitrogen for 1 to 3 minutes. Switch on both cells, and measure the reduction waves for iron, copper, cadmium, tellurium and the combined lead and thallium. (The iron and copper waves can be measured either on forward or reverse sweep.) Determine the iron, copper, cadmium and tellurium contents by comparison with a standard solution containing known amounts of these elements. If a reduction wave for lead or thallium is obtained, then these elements may be determined by using 1 M ammonium acetate - 1 M acetic acid - 0·002 M EDTA electrolyte.

DETERMINATION OF LEAD AND THALLIUM—

Approximate reduction potentials in 1 M ammonium acetate - 1 M acetic acid - 0·002 M EDTA are—

Element	Iron^{III}	Copper	Thallium	Titanium	Bismuth	Tellurium	Lead
Reduction potential, volts	0·15	0·3	0·45	0·5	0·7	1·0	1·0

To the residue in the second beaker (see "Separation Procedure") add 0·5 ml of 2 M ammonium acetate - 2 M acetic acid - 0·004 M EDTA solution. Transfer the solution to a 1-ml calibrated flask, and make up to the mark with distilled water. Transfer the solution obtained to a semi-micro polarographic cell. Prepare a reagent blank solution in a similar manner to the sample, and place it in the other cell of the differential polarograph.

De-oxygenate the solutions with pure nitrogen for 1 to 3 minutes. Switch on both cells, and measure the reduction waves for thallium and lead. The thallium wave is best measured on reverse sweep to prevent interference from the copper wave. Determine the lead and

TABLE I

TYPICAL RESULTS

Recoveries were made with the heater at 155° C (22 volts)

	Copper		Cadmium		Lead	
	Added, µg	Found, µg	Added, µg	Found, µg	Added, µg	Found, µg
Reagent blank value	—	0·1	—	0·01	—	0·01
Recovery	1·2	1·2	0·54	0·54	1·7	1·7

	Tellurium		Thallium		Iron	
	Added, µg	Found, µg	Added, µg	Found, µg	Added, µg	Found, µg
Reagent blank value	—	<0·0015	—	<0·005	—	0·2
Recovery	1·5	1·5	1·1	1·1	1·6	1·6

thallium contents by comparison with a standard solution containing known amounts of these elements. If no thallium is present, then the lead can be determined from the results obtained on the oxalic acid solutions.

SENSITIVITY OF THE METHOD—

If the impurity level is extremely low then the final volume of solution used for polarography can be reduced from 1 to 0·20 ml. The increased sensitivities will, however, be accompanied by a consequent reduction in accuracy, owing to the inevitable errors in measuring and handling this volume. It is, however, thought that this sacrifice is warranted in some circumstances.

RESULTS

Tables I and II list some typical results obtained with the methods described.

The necessity of avoiding excessive temperatures is indicated by the results shown below for the recovery of 1·5 μg of tellurium at different heater temperatures—

Temperature of heater, °C . .	258 (30 volts)	155 (22 volts)	100 (19 volts)
Tellurium found, μg	0·35	1·5	1·5

TABLE II

TYPICAL RESULTS

Sample number	Copper found, p.p.m.	Cadmium found, p.p.m.	Lead found, p.p.m.	Tellurium found, p.p.m.	Thallium found, p.p.m.	Iron found, p.p.m.
1	*	0·007	*	1·3	0·16	*
2	*	0·09	0·68	0·014	0·16	*
3	*	0·09	0·48	0·022	0·16	*

* Equivalent to the reagent blank value.

DISCUSSION OF THE METHOD

In the methods described, oxalic acid was used as electrolyte mainly because it can readily be purified by passage through ion-exchange resin. However, in different electrolytes it is possible to determine other elements having involatile bromides. For example, 0·2 M lithium chloride electrolyte was used for determining barium in the three samples described. The barium reduction wave occurs at —2·0 volts in neutral lithium chloride, and barium contents of 0·1 to 1·5 p.p.m. have been measured.

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Precise Micro-determination of Zinc and Cadmium by Photometric Titration with Disodium Ethylenediaminetetra-acetate

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A method is described for determining zinc and cadmium by weight titration with disodium ethylenediaminetetra-acetate, Eriochrome black T being used as indicator and a photometric end-point detection technique being employed. By this method, 0·6- and 0·06-mg amounts of zinc can be determined with standard errors of about 0·02 and 0·1 per cent., respectively; similar amounts of cadmium can be determined with the same precision.

A METHOD of high precision ($\pm 0{\cdot}1$ per cent. or better) for determining micro amounts of zinc and cadmium was required in connection with an investigation of the stabilities of certain halide complexes of these metal ions in aqueous solution.

Complexometric titrations are suitable for determining small amounts of metal ions, and methods for zinc and cadmium have been established.[1] For extremely low concentrations of metal, photometric end-point detection techniques have been used. Sweetser and Bricker[2] determined zinc and cadmium in solution at pH 10 by photometric titration with disodium ethylenediaminetetra-acetate (EDTA) at 222 to 228 mμ. Ten- and 1-mg amounts of cadmium were determined with standard errors of about 0·3 per cent. and 0·5 per cent., respectively; similar amounts of zinc were determined with slightly better precision. Hunter and Miller[3] determined zinc by photometric titration with EDTA and Eriochrome black T as indicator. The results for the titration of 5- and 0·5-mg amounts of zinc in the presence of various other metals showed standard deviations of 0·3 per cent. and 1·6 per cent., respectively.

In the investigation described here, a photometric method similar to that of Hunter and Miller[3] has been developed for the precise determination of amounts of both zinc and cadmium less than 1 mg. An important innovation was the use of a weight titration technique, and special attention was given to the design of the titration cell.

Many different types of titration cell have been designed for use with various commercial spectrophotometers in which the normal cell compartments have been suitably modified so that titrations can be carried out *in situ*. (An excellent review of photometric titration techniques and apparatus is given by Headridge.[4]) A disadvantage of most of these designs is that, after the initial adjustment of the optical-density scale (usually the untitrated solution is taken as zero), further checks on the reference point cannot be made. Serious end-point extrapolation errors may result from fluctuations in the reference level, particularly if these occur during the final stages of the titration. This difficulty has been resolved in the design of the titration cell described below.

EXPERIMENTAL

APPARATUS—

The titration cell is shown in Fig. 1. It was constructed from ⅛-inch and ¼-inch Perspex sheet and had a titration volume of about 25 ml, the light path being 2 cm in length. The cell was designed to stand on the normal cell-holder carriage of a Unicam SP500 spectrophotometer. Incorporation of a reference cell allowed zero checks to be made before each optical-density reading. Increments of titrant were added with the cell outside the cell compartment, and effective mixing was achieved by tilting the cell several times. This titration procedure was chosen in preference to an *in situ* method for two main reasons. First, no modifications had to be made to the cell compartment of the SP500 spectrophotometer. Secondly, since only relatively few points near the end-point were considered once the shape of a titration curve had been established, the *in situ* method offered no real advantages with respect to time-saving.

REAGENTS—

All solutions were made up with de-ionised water and stored in polythene bottles.

Standard metal solutions—Solutions containing more than 0·15 mg of metal per millilitre were prepared by dissolving a known weight of pure metal (purity greater than 99·99 per cent.) in a minimum amount of analytical-reagent grade nitric acid and making up to a known weight of solution (about 2000 g) with pure water. Solutions containing less than 0·1 mg of metal per millilitre were made up by weight dilution of more concentrated solutions.

EDTA solution—A known weight of analytical-reagent grade disodium ethylenediaminetetra-acetate, $Na_2H_2C_{10}H_{12}O_8N_2.2H_2O$ (previously dried at 80° C for 4 days) was dissolved in pure water and made up to a known weight of solution.

Buffer solution, pH 10—Analytical-reagent grade ammonium chloride (35 g) was dissolved in 285 ml of analytical-reagent grade ammonia solution, sp.gr. 0·880, and the solution was made up to 500 ml with pure water.

Eriochrome black T—The indicator (0·1 g) was dissolved in a mixture of 15 ml of triethanolamine and 5 ml of ethanol.

DETERMINATION OF WAVELENGTH FOR TITRATION—

A Perkin - Elmer 137UV recording spectrophotometer was used to determine the visible spectrum of a solution containing 19 ml of water, 1 ml of buffer solution, 6 drops of 0·001 M EDTA solution and 6 drops of Eriochrome black T indicator solution. Similar experiments were carried out on (*a*) a solution containing 19 ml of water, 1 ml of buffer solution, 6 drops of 0·001 M cadmium solution and 6 drops of indicator solution and (*b*) a solution made up as for (*a*), but containing 6 drops of 0·001 M zinc solution instead of the cadmium solution. Comparison of the spectra shewed that a maximum difference between the optical densities of the free indicator and the metal - indicator complex occurred at 640 mμ for zinc and at 660 mμ for cadmium. These wavelengths were used for the titrations.

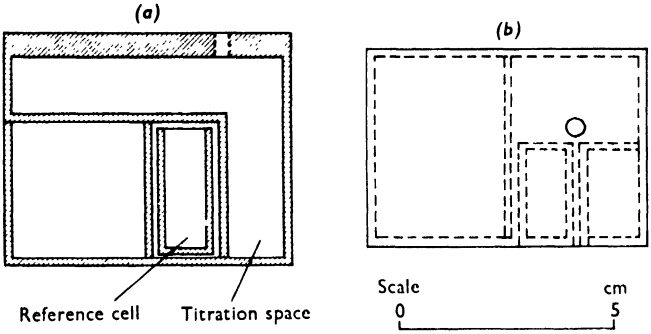

Fig. 1. Titration cell: (*a*) side view; (*b*) plan view

PHOTOMETRIC TITRATIONS—

A known weight (about 4 g) of the metal solution to be titrated was dispensed into the titration cell from a weight burette. The burette was made by drawing out the stem of a 25-ml dropping funnel to a fine jet and attaching a metal hook to the neck to facilitate weighing. One millilitre of buffer solution at pH 10 and 2 drops of Eriochrome black T indicator solution were added, together with a volume of pure water calculated to give a total titration volume of 20 ml. The indicator concentration was such that optical-density values at the end-point fell in the range 0·4 to 0·5, *i.e.*, where relative errors in optical-density readings are a minimum. EDTA solution was then added until the red solution began to turn blue. At this stage, the burette was re-weighed, and the optical density of the solution after it had been thoroughly mixed was measured at the appropriate wavelength with pure water as the reference solution. Small increments of titrant were then added, and, after each addition, the optical density of the solution was measured and the burette re-weighed. Several additions were made after the end-point had been reached. It was possible to transfer increments of titrant weighing as little as 0·005 g by allowing a small drop to form on the tip of the burette and bringing this into contact with the surface of the solution in the titration cell. A graph of optical-density readings against the weight of EDTA solution added was constructed, and the end-point was determined by extrapolation in the usual way. Optical-density readings were not corrected for volume changes, since these were negligible in the extrapolated region of the titration curves.

Four series of titrations were carried out on (*i*) 1-mg portions of cadmium, (*ii*) 0·1-mg portions of cadmium, (*iii*) 0·6-mg portions of zinc and (*iv*) 0·06-mg portions of zinc. The reagent blank value was determined by titrating a solution containing 1 ml of buffer solution, 2 drops of indicator solution and 19 ml of water.

RESULTS AND DISCUSSION

The reagent blank value was found to be 0·022 g of the EDTA solution used in the metal titrations. This value was the mean of six determinations, *viz.*, 0·022 g, 0·023 g, 0·022 g, 0·022 g, 0·022 g and 0·021 g of EDTA solution.

In the first of the two series of eight titrations of cadmium solutions (concentration 2·2452 mmoles per kg) the average weight of cadmium taken was about 0·92 mg. By taking the EDTA solution as standard, *i.e.*, assuming the exact composition of the sample of dehydrated disodium salt to be $Na_2H_2C_{10}H_{12}O_8N_2.2H_2O$,[5] the weights of cadmium found were consistently higher by about 0·2 per cent. than the weights of cadmium taken (cadmium metal = 100 per cent.). It was considered that this discrepancy was likely to be due to the non-stoicheiometry of the EDTA salt. The results of this series of titrations were used to standardise the EDTA solution a procedure that was fully justified by the results of the subsequent metal determinations. The concentration of the EDTA solution (cadmium = 100 per cent.) was found to be 0·0012609 moles per kg, the standard deviation from the mean being 0·02$_0$ per cent.

The results of the other series of titrations of cadmium and zinc are summarised in Table I.

TABLE I

THE RESULTS OF THREE SERIES OF CADMIUM AND ZINC TITRATIONS

Titrand	Concentration of titrand, mmoles per kg	Number of determinations	Approximate weight of metal taken, mg	Approximate weight of titrant required, g	Standard error in weight of metal found, %
Cadmium solution ..	0·26697	8	0·13	0·9	0·1$_5$
Zinc solution	2·0146	8	0·61	7·5	0·02$_5$
Zinc solution	0·19626	8	0·057	0·7	0·1$_5$

The main source of error in the method was found to arise from the graphical extrapolation used to locate the end-point of the titrations. This can be demonstrated by considering the titration curves in the region near the end-point. Figs. 2 (*a*) and 2 (*b*) are typical curves for the titration of 1 mg of cadmium and 0·6 mg of zinc, respectively; similar curves were obtained in the low concentration range (0·1 mg of cadmium, 0·06 mg of zinc). In all titrations the weight of titrant required could be determined to within 0·001 g of solution. Errors arising from this effect could therefore be as much as 0·01 per cent. for determinations of high concentrations of metal (when the weight of titrant was about 7 g) and approximately 0·1 per cent. for determinations of low concentrations of metal (weight of titrant about 0·8 g). These errors are of the same order as the standard errors observed in the respective concentration ranges.

An attempt to improve the precision of the determinations of low concentrations of metal by using a more dilute EDTA solution was not successful; although a higher weight of titrant was required, the dilution had the effect of producing a lower end-point slope and thereby increasing the error in extrapolation.

A feature of interest in Figs. 2 (*a*) and 2 (*b*) is the difference in the shape of the zinc and the cadmium curves. For the cadmium titration, the slope near the end-point increases with increasing weight of titrant added, whereas for zinc the slope decreases. An attempt to correlate this effect with the theoretical work of Reilley and Schmid[6] was prevented by lack of stability constant data for the metal - Eriochrome black T complexes.

Application of the method to even smaller amounts of metal, of the order of 10 μg, should be feasible, although errors of about 2 per cent. would be expected, assuming the same precision in end-point location. However, Reilley and Schmid[6] have shown that, as the concentration of indicator becomes similar to that of the metal, serious extrapolation difficulties arise; such an effect might lead to errors well in excess of 2 per cent.

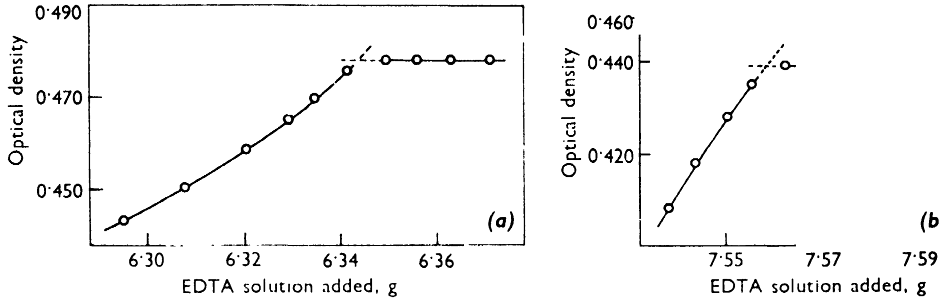

Fig. 2. Titrations of: (*a*) approximately 1 mg of cadmium; (*b*) approximately 0·6 mg of zinc

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A Spectrophotometric Method for determining Pentachlorophenol in Air

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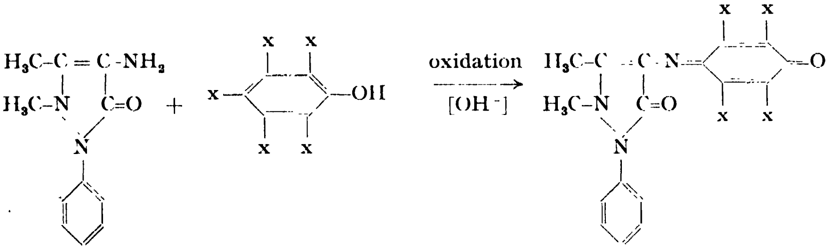
A procedure has been developed for determining pentachlorophenol with phenazone, in which ammonium persulphate is used as the oxidising agent for developing the blue colour of the phenazone - pentachlorophenol dye. By raising the pH of the reaction medium it is possible to detect the presence of lower chlorophenols and of phenol.

PENTACHLOROPHENOL and its sodium salt have recently become important owing to their fungicidal, bactericidal and herbicidal effects.[1,2,3] Reports in the literature[4,5,6] and the low maximum permissible concentration of pentachlorophenol[7] (0·5 µg per litre) have indicated its toxicity.

COLORIMETRIC DETERMINATION OF PENTACHLOROPHENOL WITH 4-AMINOPHENAZONE—

Eisenstaedt[8] observed that aromatic amines in an acid medium, or phenols in a basic medium, in the presence of an oxidising agent yield coloured compounds with 4-aminophenazone. For the basic oxidising condensation to occur, the phenols must have a free hydroxyl group in a free *para*-position, or a halogen, carboxy-group, sulpho-group or methoxy-group in the *para*-position. The reaction proceeds in accordance with the equation[8,9]—



THE PHENAZONE - PHENOL DYES—

If Emerson's equation[9] is correct, it is obvious from the reaction mechanism that the pairs of compounds listed below will form a common dye with phenazone.

Mono-derivatives—
p-Chlorophenol - phenol.

Bi-derivatives—
2,4-Chlorophenol - *o*-chlorophenol.
3,4-Chlorophenol - *m*-chlorophenol.

Tri-derivatives—
2,3,4-Chlorophenol - 2,3-chlorophenol.
3,4,5-Chlorophenol - 3,5-chlorophenol.
2,4,6-Chlorophenol - 2,6-chlorophenol.

Tetra-derivatives—
2,3,4,5-Chlorophenol - 2,3,5-chlorophenol.

Penta-derivatives—
2,3,4,5,6-Chlorophenol - 2,3,5,6-chlorophenol.

Phenol and the lower chlorophenols give an orange or red colour with 4-aminophenazone; pentachlorophenol gives a blue colour, which has been erroneously described as green.[10] Of the chlorophenols, only 2,3,5,6-tetrachlorophenol gives the same blue colour, and this is evident from the structural formulae of the phenazone dyes. The absorption curve for the phenazone - pentachlorophenol dye is shown in Fig. 1, and the stability of the dye is indicated in Fig. 2.

The phenazone - pentachlorophenol dye is extremely soluble in ether and benzene, less soluble in chloroform and insoluble in light petroleum. The most convenient solvent for extracting the dye is benzene, as the red colour formed in the presence of an excess of 4-aminophenazone is insoluble in it, and it is less volatile than is ether.

OXIDISING AGENT—

The colour reaction of 4-aminophenazone with phenolic substances is an oxidation condensation. Emerson used potassium ferricyanide in a basic medium and ferric chloride in an acid medium as oxidising agents for the reaction, and others[10,11,12,13,14,15] also used potassium ferricyanide. This reagent is, however, unstable; half an hour after a reagent solution had been prepared the presence of ferrocyanide could be shown by Tananajev's drop reaction.[16] Although no significant changes in the concentration of the solution were observed, changes in the colorimetric properties occurred; a more stable reagent was therefore sought that would be colourless in the reduced form. From the compounds investigated, ammonium persulphate and sodium persulphate were the most suitable.

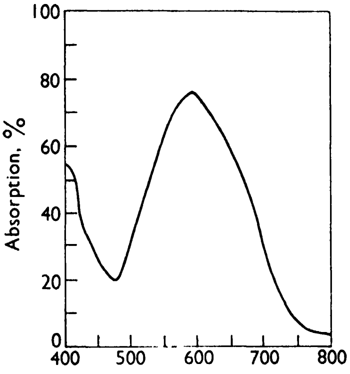


Fig. 1. Absorption spectra of the blue 4-aminophenazone - pentachlorophenol dye

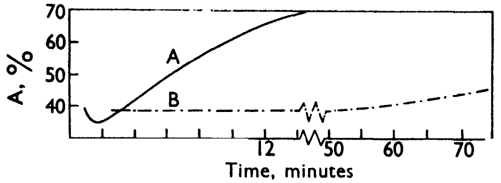


Fig. 2. Stability of the 4-aminophenazone - pentachlorophenol dye in: curve A, water; curve B, benzene

pH OF THE REACTION MEDIUM—

The phenazone - phenol dyes are formed in a basic medium and the colour intensity is dependent on pH of the solution; this can be explained by the fact that the amino-group of the 4-aminophenazone is polarised by the reaction medium. It was observed that the reaction did not proceed quantitatively if the pH of the solution was not high enough. If a mixture of phenolic compounds having different substituents in the *para*-position is available, the electro-positivity of the hydrogens of the 4-aminophenazone amino-groups can be decreased by control of the pH of the solution, so that the 4-aminophenazone reacts with the derivative having, in the given medium, the greatest inductimetric effect on the $\geqslant$C–X bond in the *para*-position.

At pH 7 to 7·5 the blue colour produced by pentachlorophenol and 2,3,5,6-tetrachlorophenol can be extracted into the benzene phase. The lower chlorophenols and 2,3,4,5-tetrachlorophenol react at pH 8 to 8·5 to give a red colour, and phenol reacts at about pH 10 to give an orange-yellow colour. Pentachlorophenol does not react with 4-aminophenazone at above pH 8·5, and lower chlorophenols and other phenazone-active compounds, except phenol, do not react at above pH 9 to 9·5. The presence of lower chlorophenols or phenol in a sample can therefore be detected by raising the pH of different solutions of the sample. Only the blue colour produced by pentachlorophenol or 2,3,5,6-tetrachlorophenol can be quantitatively extracted into benzene; the red phenazone dye is insoluble, and the dyes produced by the lower chlorophenols, except 2,3,5,6-tetrachlorophenol, are only partly soluble.

The colour intensity cannot subsequently be increased by adjusting the pH of the solution since, as already mentioned, the 4-aminophenazone residue is condensed and forms a red dye. The reaction is extremely sensitive to pH, and the timing of the operations in the procedure is important.

Distillates of biological materials, especially urine, have widely different pH values; this is not easy to overcome by the use of buffer solutions without affecting the colour intensity or raising the lower limit of the determination. For these reasons the optimum pH value is adjusted colorimetrically.[17]

The determination of pentachlorophenol in the atmosphere poses a similar problem. If, as well as pentachlorophenol, other compounds, *e.g.*, basic compounds, sulphur dioxide and hydrogen sulphide, that can affect the pH of the absorbing solution are present in the atmosphere, the optimum amount of sodium carbonate must be ascertained experimentally before the determination is carried out.

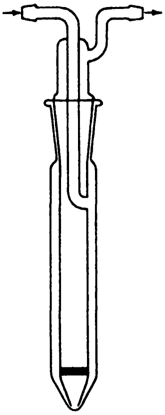


Fig. 3. Bubbler

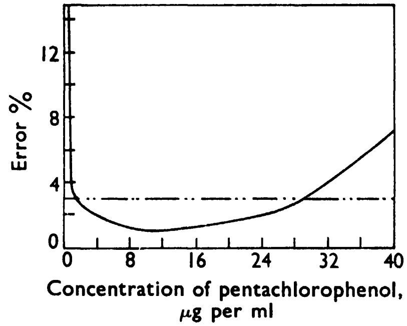


Fig. 4. Error of the method

METHOD

REAGENTS—

Sodium carbonate solution, 1 *per cent. w/v.*

4-Aminophenazone solution, 0·2 *per cent. w/v*—Prepare from 4-aminophenazone purified chromatographically by passing a solution of it in light petroleum through a column of alumina. The impurities remain on the column and the 4-aminophenazone passes through. Crystallise the 4-aminophenazone (m.p. 108° to 108·5° C) from the light petroleum, and dry the crystals in a vacuum desiccator. Store the solution in a refrigerator.

Ammonium persulphate solution, 8·4 *per cent. w/v*—Store the solution in a refrigerator.

Benzene—Analytical-reagent grade.

APPARATUS FOR TAKING SAMPLES FROM THE ATMOSPHERE—

The apparatus consists of two sintered-glass bubblers (see Fig. 3) arranged in series, a flow meter, a T-tube and a vacuum pump. The flow meter is connected between the bubblers and the pump, and the free end of the T-tube is used for controlling the rate of flow.

The advantages of using bubblers of the type[18] shown in Fig. 3 are that, as all the absorbing liquid is above the sintered disc, maximum contact is obtained between the air stream and the absorbing liquid, and the loss of absorbing liquid is small.

PROCEDURE—

Put 5 ml of distilled water into each of the bubblers, and draw the air sample through at a rate of 2 litres per minute for 10 minutes.*

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* If the air sample is acidic, quantitative sampling can be effected by adding to the contents of the absorber the necessary (experimentally determined) amount of sodium carbonate.

Then add to the contents of each bubbler sufficient sodium carbonate solution to bring the pH to 7·4. (For an accurate determination, the required amount of sodium carbonate solution is estimated by the colorimetric titration method; if speed is more important than accuracy, the error due to the salt effect—3 to 8 per cent.—is ignored, and the pH is adjusted to 7·4.) Add 0·25 ml of 4-aminophenazone solution to 5 ml of the sample solution, and mix thoroughly; then add 0·5 ml of ammonium persulphate solution, and again mix thoroughly. Ninety seconds after the addition of the ammonium persulphate solution, extract the blue dye by shaking with 2 ml of benzene; one extraction for 10 to 15 seconds is sufficient. Measure the optical density at 589 mμ against pure benzene.

PREPARATION OF CALIBRATION CURVE—

Two calibration curves should be prepared, one for low and one for higher concentrations of pentachlorophenol. Place in pairs of test-tubes equal amounts of pentachlorophenol in the range 0 to 30 μg per ml. Develop the blue dye, as described above, extract each solution with benzene, and measure the optical densities of the benzene extracts in 0·5-cm cells. Then combine the pairs of extracts containing equal amounts of pentachlorophenol, and measure the optical densities in 1·0-cm cells.

Check the calibration curves twice monthly.

PREPARATION OF STANDARD ATMOSPHERE—

To check the method it was necessary to prepare a standard atmosphere, and for the purpose a 850-litre dust chamber was used.[19] A fine dust of pentachlorophenol was introduced into the chamber in an inert carrier; mixing was by a fan on the bottom of the chamber. The physical constants of the dust and its ultraviolet spectrum were determined, and the amount of pentachlorophenol in the dust was then adjusted gravimetrically.

The atmosphere in the chamber was controlled gravimetrically and checked by a chemical method. Gravimetric control was possible as the ratio of pentachlorophenol to carrier was 1 to 500 to 1 to 1000. Simultaneously, a sample of dust was collected on a diaphragm filter and with a bubbler. The dust on the filter was weighed, and the concentration of pentachlorophenol was calculated from the total weight of dust. The concentration of pentachlorophenol in the dust was also determined by Deichmann and Schafer's method,[20] and in no instance was there any significant difference between the results. The maximum difference was ±3·5 per cent.; a possible explanation is that the determination was carried out in the absence of materials that could affect the analytical procedure.

DISCUSSION OF THE METHOD

When the sample is taken at the correct rate, the second bubbler will not contain any pentachlorophenol. For high concentrations of pentachlorophenol it is advisable to use bubblers without sintered-glass discs, as a precipitate is formed and the sample solution must be diluted; if a further sample is to be taken, less air should be sucked through the bubblers.

To avoid taking samples having concentrations below the lower limit of the determination, two pairs of bubblers connected in parallel can be used. Then, if necessary, the benzene extracts from the two first bubblers can be combined, as can those from the two second bubblers.

INTERFERENCES—

2,3,5,6-Tetrachlorophenol alone interferes, as it also forms a blue dye; all other chlorophenols form a red dye under different conditions. Only the dyes formed by pentachlorophenol and 2,3,5,6-tetrachlorophenol can be extracted quantitatively by benzene, and hence a 100 per cent. excess of the other phenols would not affect the determination of pentachlorophenol.

SENSITIVITY—

The method will detect down to 0·15 μg of pentachlorophenol per litre of air, and the lower limit for the quantitative determination is 0·25 μg of pentachlorophenol per litre of air; the error of the method is shown graphically in Fig. 4.

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Determination of Tocopherols in Plant Tissues

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Leaves or other tissues are extracted with cold acetone and light petroleum. The washed extract, containing the lipids, is concentrated to small volume and applied to treated chromatographic paper. The chromatogram is developed in two dimensions. α-Tocopherol is observed under ultraviolet irradiation as an absorbent spot in the middle of the chromatogram. α-Tocopherol and other tocopherols, if present, are cut out and determined with ferric chloride - bipyridyl reagent.

The technique is a sequel to that described by the Analytical Methods Committee of the Society for Analytical Chemistry in 1959.

FORMER METHODS

EARLIER methods for determining tocopherol or "vitamin E," as applied to plant tissues, usually had two errors. First, tocopherol was lost—enzymically[1] or chemically—through maceration of the sample before extraction or through using heat, alkali or destructive adsorbents. Second, reducing substances other than tocopherol were counted as vitamin E, including coenzyme Q_{10} (ubiquinone) reduced during chromatography on Florex,[2] lipids such as "u4" and "u5" (see Booth[3]) and artifacts found in solvents or eluted from chromatographic columns.[4]

Tocopherols were usually separated from impurities and from one another by column chromatography, a procedure corresponding to one dimension alone on two-dimensional paper chromatography. The tocopherols, being invisible, must be collected from the columns in large volumes of eluate that then contain other substances. Even if the eluate be divided, each fraction must be tested by unspecific colorimetry, a tedious procedure that multiplies errors due to blanks and artifacts.

THE PROPOSED METHOD

The method described here is based on (*a*) the procedure used for carotenoids and other phyllolipids[5] in which the lipids are quickly extracted from small samples into acetone and light petroleum, and then (*b*) two-dimensional paper chromatography as described by Green, Marcinkiewicz and Watt.[6] The latter, adsorption chromatography with subsequent reversed-phase or partition chromatography, further developed by the Analytical Methods Committee (A.M.C.),[7] has now been so modified that plant extracts may be chromatographed without previous saponification or "purification."[3] The phyllolipids are displayed on one sheet with α-tocopherol in the middle, whence it is removed for assay with bipyridyl. The time from weighing a sample to reading the optical density is about 5 hours, and several duplicate sets can be completed in a day.

The method has been used for leaves, stems, flowers, fruits and roots. In most plant tissues other than seeds the predominant tocopherol is the α. Certain leaves[3] and fruits contain γ- and δ-tocopherols, which develop separately on chromatograms. Work in progress suggests that the method could form a basis for determining other lipids that appear on chromatograms.[3]

SOLVENTS AND APPARATUS—

Solvents—Light petroleum freed of fluorescing substances by passage through aluminium oxide. Other solvents purified by accepted procedures.

Separating funnels of about 300 ml capacity. No stopper is needed; and a smooth neck having a spout is recommended.

Whatman No. 4 filter-paper "for chromatographic purposes" (32 cm × 22 cm) with machine direction along the short dimension. After being dipped in the zinc carbonate solution,[7] these papers curl less and dry more evenly than if the fibres run predominantly downwards. This orientation also minimises chromatographic time in the slower, second, dimension. Fan out the papers, lay a ruler across the lower stepped edges, and pencil a line to mark each sheet at E, Fig. 1. Insert edge T of each sheet into a wide paper-grip, off centre to permit good draining, dip the paper in zinc-ammine solution[7] and hang the sheets to dry.[7] Check the evenness of the coating under ultraviolet irradiation, and store the dry papers under a paperweight.

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Frames[7,8]—Standard frames, 20 cm (8 inches) square, are improved by reducing the rods to 18 cm long, and drilling fresh holes nearer the corners of the plates. Corresponding holes in the paper appear in Fig. 1. Although the papers are larger than the frames, the overhangs cause no inconvenience.

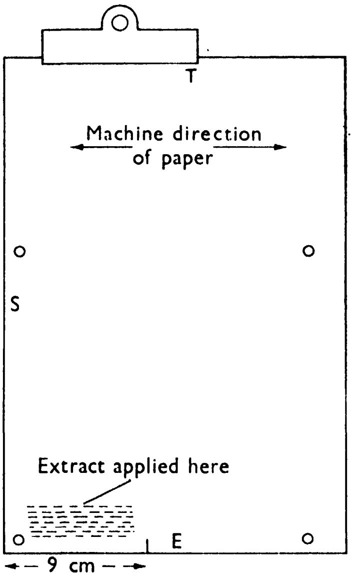

Fig. 1. Chromatographic paper. Extract is applied to the treated paper over the indicated area. Scale: one-fifth full size

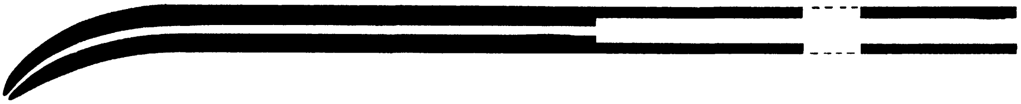

Fig. 2. Self-filling curved micropipette. Length of capillary about 7 cm, of wide part about 10 cm; capacity about 50 μl. The tip is rounded and well polished

WORKING DIRECTIONS—

Extraction—Weigh, into a heavy 50-ml beaker, about 300 mg of fresh leaf, taking small snippings from many leaves, or a larger amount of fruit, etc., taking carefully cut wedges. Extract the sample with acetone[5] and then with light petroleum (boiling range 30° to 40° C).[3,9] Wash the acetone from the petroleum with water by the automatic drip-wash procedure.[10,11] If an emulsion forms while an extract is being washed, and twirling the separator fails to disperse it, add more light petroleum and wash slowly. Transfer the extract to a wide-necked conical flask, and concentrate, with the aid of a stream of nitrogen and a water bath at 60° C, to about 1 ml.

Application of extract to paper—Fit a chromatographic paper under a weight with edge E overhanging the bench by about 5 cm.[12] Apply the extract to the paper by sliding the filled pipette[3] (see Fig. 2) across the width of the setting area. Repeat the additions from the pipette until all the solution has been applied to the paper evenly over an area about

7 cm × 2 cm, leaving 1·5-cm margins as in Fig. 1. A bluish green material that survives several rinses of the flask may be ignored, although complete transfer to paper can be effected with benzene. (Light petroleum, boiling range 30° to 40° C, evaporates quickly from the paper, but when other solvents or large pipettes are used an evaporator is useful.[12])

Development of the chromatograms—Mount the papers on a frame, and include a paper without extract for the blank reading. Place the frame in a lined chromatographic tank at least 40 cm high, with paper edges E dipping into a solution of 1 per cent. of acetone in light petroleum, boiling range 40° to 60° C[3] (2 per cent. for rich extracts or re-chromatographed solutions). After 1½ hours the lipids should be spread in bands up the "corridor." Remove the frame, coat the clear three-fifths of the papers with medicinal (liquid) paraffin,[7] and place the assembly in a second chromatographic tank with paper edges S dipping in methanol. Fig. 3 shows the condition of a chromatogram after 1½ hours.

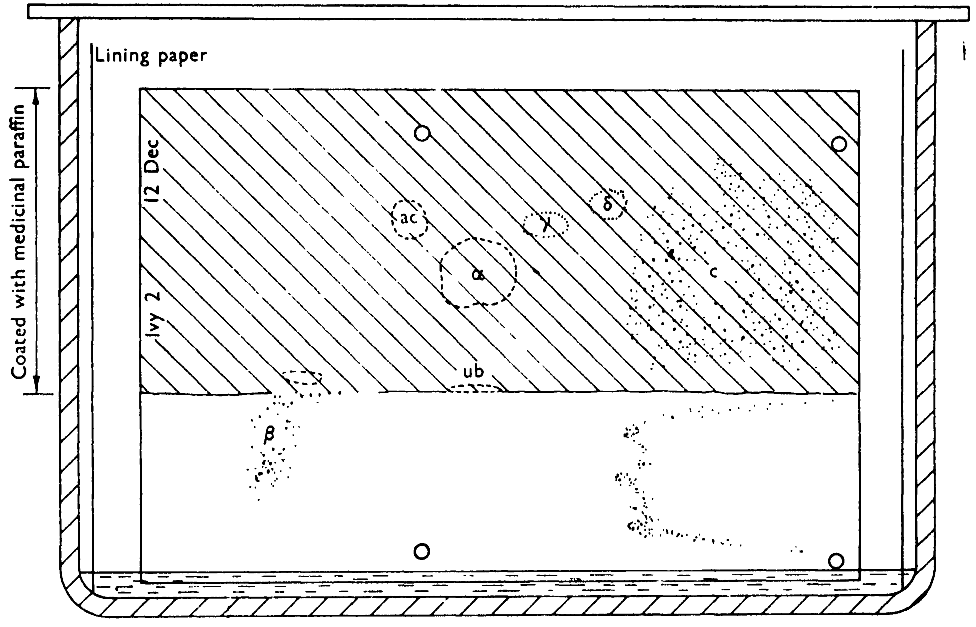

Fig. 3. Appearance of chromatogram after development from methanol (whose depth is exaggerated for clarity). Dotted areas represent pigments; areas enclosed by dotted lines represent lipids that absorb ultraviolet irradiation: β, β-carotene; ub, ubiquinone; α, α-tocopherol; γ, γ-tocopherol; δ, δ-tocopherol; c, carotenoids and chlorophylls; ac, fluorescing material from unpurified acetone. Several other lipids are also usually present

Colorimetric determination of tocopherol—Dry the papers for 5 minutes in a current of air. Because even drying is essential,[13] a jet of gas is unsuitable. Remove the papers from the frame, examine them under an ultraviolet lamp, cut out the tocopherol spots[7] and blanks, and cover each with 3·5 ml of a 0·02 per cent. solution of 2,2′-bipyridyl in ethanol.

From a syringe pattern or "walking stick" micropipette[14] add 0·5 ml of ferric chloride solution (0·1 per cent. w/v of $FeCl_3.6H_2O$ in ethanol) to the blank. Measure the optical density at 520 $m\mu$, then repeat the procedure for the tocopherols, as described by the A.M.C.[7] For δ-tocopherol allow at least 3 minutes for completion of the colour reaction. Extremely weak spots of a tocopherol from duplicate papers may be pooled to give a satisfactory optical density.

NOTES ON PROCEDURE

SIZE AND NATURE OF SAMPLE—

Production of strong spots demands as much extract as practicable per paper, yet too much leads to crowding of lipids, making isolation of tocopherols uncertain. Best results are obtained when chlorophylls run 10 cm in the first dimension (see Fig. 3). The optimum weight of leaf sample is about 40 mg of dry matter, but larger samples of pale green leaves may be used. Fruits, roots and stems are so low in lipids that larger samples are practicable and necessary. On the other hand, slowly growing leaves may be so rich (see Table I) that a portion of the extract suffices.

TABLE I

α-TOCOPHEROL CONTENTS OF PLANT TISSUES

			α-Tocopherol found	
Species	Tissue	Month	μg per g of dry matter	μg per g fresh weight
Taxus baccata (yew)	Leaf	Jan.	1450	480
Lamium purpureum (red deadnettle)	Leaf	Feb.	210	40
Psalliota campestris (mushroom)	—	Feb.	5	0·5
Dactylis glomerata (cocksfoot grass)	Leaf	Apr.	110	20
Sultana	Fruit	Apr.	8·5	7
Populus nigra (black poplar)	Leaf	July	340	115
Typha angustifolia (bulrush)	Leaf	Aug.	1120	290
Bryonia dioica (bryony)	Leaf	Sept.	900	150
Pteridium aquilinum (bracken)	Leaf	Oct.	450	150
Rubus idaeus (raspberry)	Leaf	Oct.	1400	580
Trifolium repens (white clover)	Leaf	Nov.	260	45
Helianthus tuberosus (artichoke)	Tuber	Dec.	15	3

When the nature of the material demands samples so large that an extract would overload a paper, use a portion or share the extract over two or more papers.[3] When several grams of material are extracted, a Waring blender is useful, although mechanical aids are avoided for small samples because they consume more time than extraction in beakers.[15]

In analysing materials with moisture contents <60 per cent., add a few drops of water before beginning the extraction.

LOSS BY OXIDATION—

When leaves are damaged tocopherols are lost.[1] Hence extraction must be carried out promptly after sampling. Loss by oxidation of adsorbed tocopherols exposed to air is also possible. Therefore limit the papers on a frame to three, and place loaded papers in the first tank without delay.

CHROMATOGRAPHY—

Methanol is used as mobile phase for reversed-phase chromatography because it migrates quickly. Methanol evaporates from the tank, and water from the air may condense into it. Counter these effects by only momentarily removing the lid when transferring papers, by topping up daily, and by renewing with freshly-distilled methanol weekly. The last is advisable because stale methanol destroys tocopherol.

For good chromatograms, extracts must be applied evenly; uneven application is shown by peaks in the corridor. If chromatographic spots are distorted or not separated, perhaps too much extract was applied. Try smaller samples or apply the extract on a wider area. Wide corridors, however, leave little space for second dimensional development. It is not advisable to move E (Fig. 1) beyond 10 cm from edge S.

Extracts may be applied to paper with a micrometer syringe. This is accurate, but even application is difficult. Alternatively, cut a slit 1 cm long at E (Fig. 1) and another 7 cm to its left, bend the paper flap, and dip it repeatedly into the extract in a porcelain combustion boat. Drive lipids out of the flap's edge with acetone as in the spiral technique. The boat scheme produces excellent chromatograms, but quantitative application is tedious.

The chromatographic times are not critical and may be exceeded; indeed, longer times may be necessary, especially in the second dimension and at temperatures >20° C.

SPIRAL TECHNIQUE—

When much tocopherol is needed for identity tests, roll three zinc-treated Whatman No. 31 (extra thick) papers into loose scrolls around the short dimension. Stand a coiled paper for 3 seconds in a litre beaker holding strong extract 2 mm deep. Invert the paper to allow solvent to evaporate while other papers take their turn, and repeat until the papers are loaded. Add acetone for the last dippings, then briefly dip each paper twice in a shallow layer of acetone to carry lipids from the edge to prevent their dissolving in the chromatographic tank. While the scrolls stand in the first tank, lipids should separate chromatographically into wavy bands. Elute those required into acetone, transfer to light petroleum, boiling range 30° to 40° C, apply to one paper and re-run in two dimensions.

IDENTIFICATION OF TOCOPHEROLS—

The most widespread tocopherol is α-tocopherol. Typically, it appears near the middle of a chromatogram (see Fig. 3), but as up to 40 phyllolipids may be present,[3] hints are offered on its recognition. (1) A fluorescent laboratory coat may interfere with the recognition of ultraviolet-absorbing spots, and should be removed before chromatograms are scanned. (2) On chromatograms of extract from dark green leaves α-tocopherol is so conspicuous[16] that it is recognised unambiguously. (3) In preliminary tests, unpurified acetone, giving a spot that fluoresces blue beyond α-tocopherol (Fig. 3), may help to locate the tocopherol. Ubiquinone, nearly always present,[3] also acts as pointer. (4) Spray a chromatogram with ferric chloride - bipyridyl (full strength recommended by the A.M.C.[7]) or dianisidine reagent.[3,7] (5) Observe suspect spots, and spots of authentic α-tocopherol, under an ultraviolet lamp with different filters. Tocopherols quench the fluorescence under each, but non-tocopherols may disappear under longer wavelength irradiation.[6] Elute[17] the selected spot from at least two chromatograms into methanol, transfer to light petroleum and re-chromatograph from methanol in one dimension to remove traces of paraffin. Elute the suspected tocopherol (now close to the solvent front) into ethanol, and observe the spectral absorption maximum.[18] (7) Compare the eluted "tocopherol" with the authentic tocopherol by mixed chromatography. (8) Most known tocopherols can be separated by gas - liquid chromatography,[19,20] and observation of relative retention volumes may be used to confirm identities.

TOCOPHEROL AND THE BLANK—

The blank reading is advisedly taken first to check that the ferric chloride solution is in order. The importance of controlling the magnitude of the blank has been discussed elsewhere.[7,13] With the reagent concentration and technique described here, (*a*) the optical density of the blank should be <0·030, and, given reasonable exclusion of light, can be much lower; also (*b*) the effect of dry paper on the magnitude of the blank is small. Therefore a paper blank need only be included infrequently, but a paperless blank must be read at each determination, when allowance can be made for the effect of the paper.

SAPONIFICATION AND OTHER PRE-TREATMENT—

When fat is present, as in seeds or animal tissues, some purification of the extract before chromatography may be desirable, though pre-treatment will lead to some loss of tocopherol. If chromatograms are distorted, saponification may bring some improvement. Follow the instructions given by the A.M.C.[7] (p. 361), or by Booth[10] (p. 60). If a long-necked flask is used, a condenser is unnecessary. Sterols can be frozen out from solution.[7] Treatment of the extract with Decalso[21] removes interfering substances, but may also remove some tocopherol.[4] Floridin earth, the "classical" adsorbent for purifying tocopherols, is best avoided.[4] The use of larger papers or the spiral technique may help to obviate the need for pre-treatment.

ACCURACY AND PRECISION

Over 95 per cent. of the tocopherol is removed from leaves in two extractions, and extraction is complete when all pigment is removed.

When 25 μg of α-tocopherol were added to portions of a leaf extract and taken through all stages, the recovery was 95 ± 1·1 per cent. Some α-tocopherol, a labile substance, is inevitably lost during manipulations by any technique. Claims of 100 per cent. recovery should not be accepted uncritically. Fat solvents, especially after percolation through adsorbents, contain reducing substances.[4] These, unless removed by paper chromatography, will be included in the bipyridyl test and may lead to spuriously high recoveries.

The standard deviation of one determination, estimated from 144 duplicate determinations of the α-tocopherol contents of plant tissues, ranged from ±0·3 at 2 p.p.m. fresh weight to ±10 at 300 p.p.m. The coefficient of variation of the mean of duplicate determinations was about ±7 per cent.

EXAMPLES

α-Tocopherol contents of samples of plant tissues, determined in duplicate by the proposed method, are shown in Table I. Other examples have been given by Booth and Hobson-Frohock.[16]

I thank Mr. M. P. Bradford for technical assistance and helpful suggestions.

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The Analysis of Geranium Oil by Gas Chromatography

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The separation of the constituents of geranium oil by means of gas chromatography has been studied. Five stationary phases were tested and polyethylene glycol 400 was found to be the most effective. The peaks were classified by noting the changes in the chromatogram that occurred when the oil was treated with group reagents. Individual peaks were identified by their retention times. Chromatograms of oils from Algeria, Morocco, Kenya and Réunion were obtained. The patterns produced by the North African oils were found to exhibit certain characteristics allowing them to be distinguished from the Bourbon oils from Kenya and Réunion. A series of oils obtained from plants at various stages of maturity were examined, and differences between them were readily detected.

GERANIUM oil is an important and valuable raw material for the manufacture of perfumes and has been the subject of many investigations. Nearly thirty compounds have been reported in oils from various sources.[1] Since most of the many constituents must contribute to the aroma of the oil, it is not surprising that the analytical constants normally used in the analysis of essential oils can be correlated with the source and quality of geranium oils only in a broad manner.

A rapid and reliable analytical method capable of giving results that could be interpreted in terms of quality and origin would clearly be useful. In seeking such a method, gas chromatography is the obvious choice.

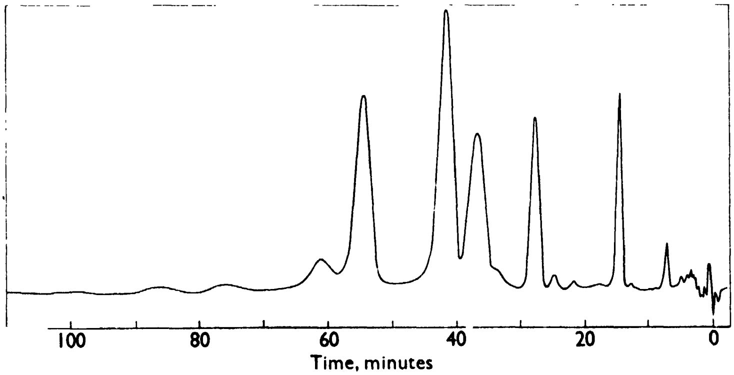

Fig. 1. Chromatogram of Kenya geranium oil with propylene sebacate polyester as stationary phase

Chromatograms of geranium oils have been published by Bayer, Kupfer and Reuther,[2] who used a 9 + 1 mixture of silicone grease and lithium hexanoate as the stationary phase and firebrick as the support, by Teitelbaum,[3] who did not specify the composition of his column, and by Suffis and Dean,[4] who used 25 per cent. of Carbowax 1540 on Chromosorb-W. These chromatograms contained only about ten peaks, none of which was separated completely from adjacent peaks.

The objects of the work described here were to discover a stationary phase capable of resolving all of the major constituents of the oil, to identify the peaks and to demonstrate the utility of the method in determining the origin of the oil and assessing its quality.

The testing of the stationary phases, and the identification of peaks, were carried out on a geranium oil produced in Kenya from plants obtained by the propagation of cuttings of Bourbon geraniums from Réunion.

EXPERIMENTAL

STATIONARY PHASES—

Since geranium oil consists largely of alcohols and esters, a polar stationary phase was indicated. Preliminary experiments showed that propylene sebacate polyester would not separate citronellol from nerol at the optimum temperature for the analysis of geranium oil, and could only partly separate citronellol from citronellyl acetate, and geranyl formate from citronellyl acetate. As all of these compounds, except nerol, were known to be important constituents of geranium oil, this stationary phase was obviously unsuitable. A more polar stationary phase, namely polyethylene glycol 400, was tried.

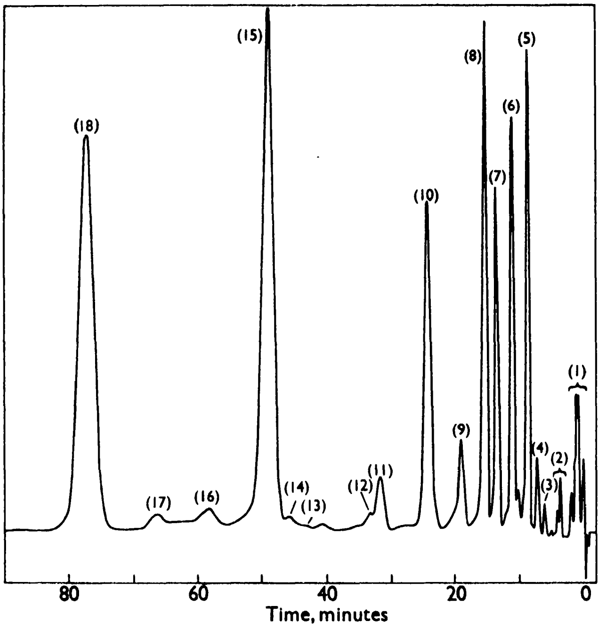

Fig. 2. Chromatogram of Kenya geranium oil with polyethylene glycol 400 as stationary phase. Peaks: (1) hydrocarbons; (2) ethers; (3) hydrocarbon; (4) hydrocarbon + menthone; (5) isomenthone; (6) hydrocarbon; (7) linalol; (8) citronellyl formate; (9) citronellyl acetate; (10) geranyl formate; (11) aldehyde; (12) geranyl acetate; (13) ester; (14) ester; (15) cintronellol; (16) nerol; (17) ester; (18) geraniol

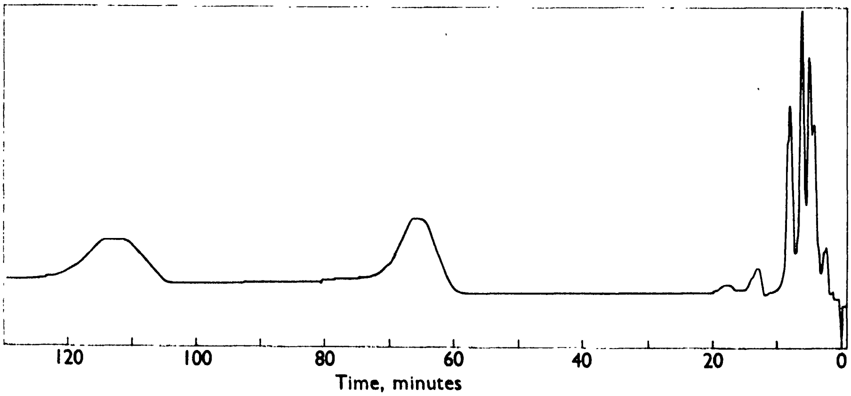

Fig. 3. Chromatogram of Kenya geranium oil with diglycerol as stationary phase

Chromatograms obtained by means of propylene sebacate polyester (Fig. 1) contained only 22 peaks, whereas polyethylene glycol 400 (see Fig. 2) gave about 34 peaks, and was able to separate all of the substances mentioned above.

It is always desirable to confirm the results obtained on one stationary phase by repeating the examination on another. Polyethylene glycol, however, is one of the two most polar of the common stationary phases, and less polar substances were evidently unsuitable.

The other common, highly polar, stationary phase is diglycerol, which, like polyethylene glycol, contains both ether and hydroxyl groups, although diglycerol is predominantly hydroxylic whereas in polyethylene glycol the ether groups predominate. A column containing diglycerol was prepared, but the chromatograms obtained from this column contained only 18 peaks (see Fig. 3).

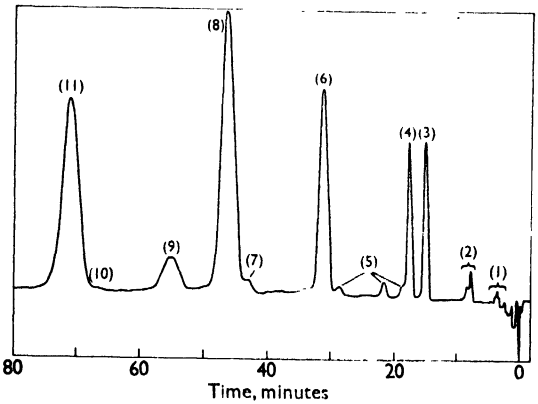

Fig. 4. Chromatogram of Kenya geranium oil with polyethylene glycol salicylate as stationary phase. Peaks: (1) hydrocarbons; (2) ethers; (3) linalol; (4) isomenthone; (5) terpenes; (6) hydrocarbon with citronellyl formate; (7) citronellyl acetate; (8) citronellol + geranyl formate; (9) nerol; (10) geranyl acetate; (11) geraniol

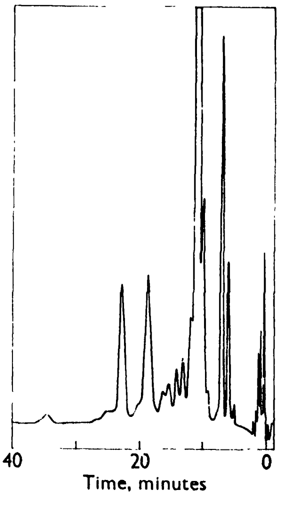

Fig. 5. Chromatogram of hydrocarbon fraction from Kenya geranium oil with polyethylene glycol 400 as stationary phase

It was thought that esters prepared from polyethylene glycol and acids containing polar substituents might have the desired properties. The salicylate and 3,5-dinitrobenzoate were prepared. The dinitrobenzoate, however, gave extremely short retention times and poor separations; it was not studied any further. The chromatograms given by the salicylate column (see Fig. 4) were also rather disappointing, but they contained rather more peaks than those given by propylene sebacate polyester, and it was decided to use a salicylate column to check the results obtained with polyethylene glycol.

IDENTIFICATION OF CONSTITUENTS—

The functional groups present in the oil were identified by comparing a chromatogram of the original oil with chromatograms of samples of the oil treated with reagents specific for a given group.

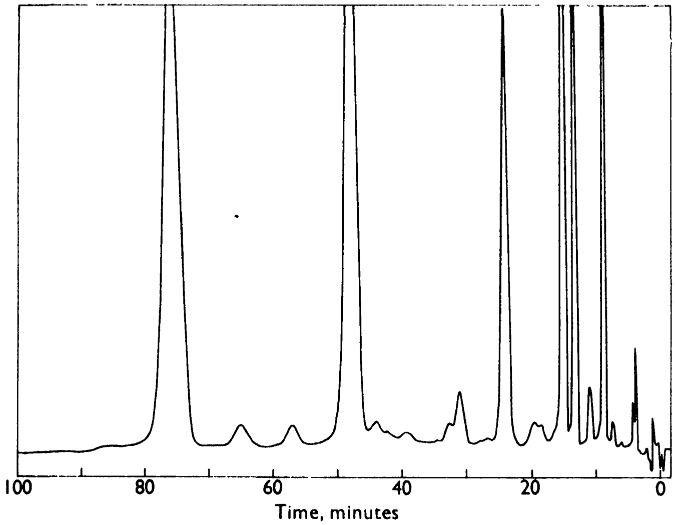

Fig. 6. Chromatogram of oxygenated compounds from Kenya geranium oil with polyethylene glycol 400 as stationary phase

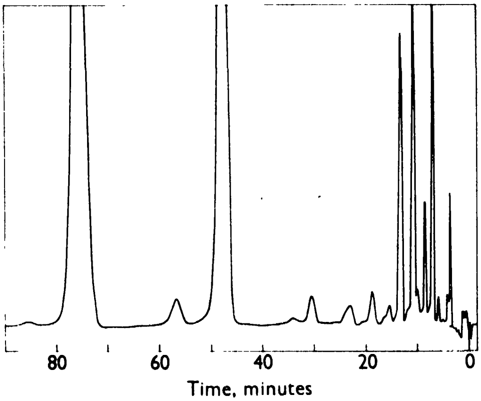

Fig. 7. Chromatogram of Kenya geranium oil after hydrolysis; stationary phase, polyethylene glycol 400

The oil was separated into a hydrocarbon fraction and an oxygenated fraction by Kirchner and Miller's method.[5] The chromatograms obtained from these fractions are shown in Figs. 5 and 6.

To check the efficiency of this separation, the infrared spectrum of the hydrocarbon fraction was determined. The only intense band was that due to the stretching of the C–H bonds (2900 cm^{-1}). The absence of strong bands in other regions indicates that no appreciable amounts of oxygenated compounds were present. The spectrum of this fraction and the chromatographic behaviour of its major constituent were similar to those of caryophyllene, but not identical with them. It is probable therefore that the major hydrocarbon was a sesquiterpene structurally related to caryophyllene.

Esters were hydrolysed with alcoholic potassium hydroxide, alcohols were acetylated with acetic anhydride and carbonyl compounds were removed by hydroxyammonium chloride. Aldehydes were removed by means of dimedone. Chromatograms of the treated oils obtained with the polyethylene glycol column are shown in Figs. 7, 8, 9 and 10.

A portion of the oil was shaken with water and another with sodium carbonate solution. The chromatograms of these portions were identical with that of the original oil. It appears, therefore, that no appreciable amount of water-soluble or acidic compounds was present in the oil.

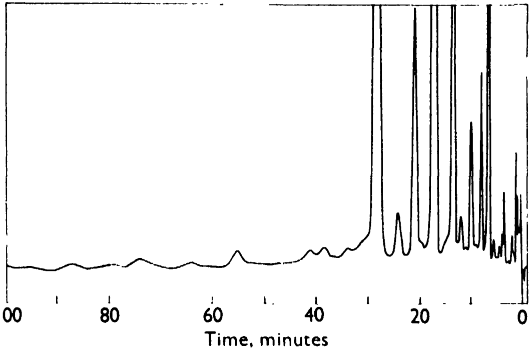

Fig. 8. Chromatogram of Kenya geranium oil after acetylation; stationary phase, polyethylene glycol 400

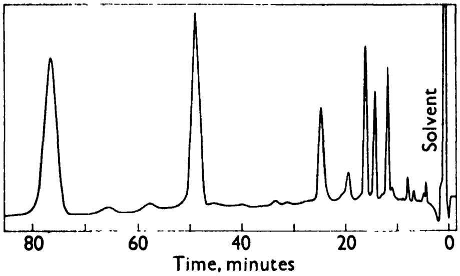

Fig. 9. Chromatogram of Kenya geranium oil after treatment with hydroxyammonium chloride reagent; stationary phase, polyethylene glycol 400

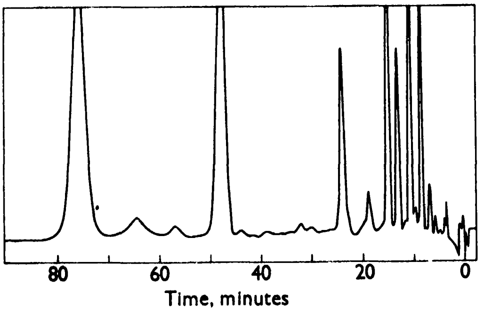

Fig. 10. Chromatogram of Kenya geranium oil after treatment with dimedone; stationary phase, polyethylene glycol 400

All but two small peaks were classified by this method. These two substances appeared to contain oxygen, since they could not be eluted from silica gel by n-hexane, but they did not react with any of the reagents. This suggests that they might be ethers. The polyethylene glycol column was unable to separate the first of these compounds from cineole, but they were separated completely by polyethylene glycol salicylate. Naves, Lamparsky and

Ochsner[6] have reported the presence of *cis* and *trans* (—) 4-methyl-2-(2-methylprop-1-enyl)-tetrahydropyran in geranium oil, and the peaks could be due to these compounds.

Owing to the many peaks in chromatograms of geranium oil and their proximity to each other, it was impossible to use an internal marker in order to identify individual peaks by their relative retention volumes unless a compound with a greater retention volume than any of the constituents of the oil was chosen. Several compounds were tested on the polyethylene glycol column, but the only ones that could possibly be used had retention volumes at least 50 per cent. greater than that of geraniol and tended to give skew peaks. It was found, however, that after the apparatus had been running for about an hour the retention time of any peak was extremely reproducible. Values determined on different days differed considerably, but this does not invalidate the use of retention times for identification purposes, provided that the sample and an authentic specimen of a constituent are run consecutively within a short time.

Authentic specimens of compounds whose presence in the oil was suspected were run alternately with the oil. Their retention times and the retention times of the corresponding peaks in the oil were determined. These retention times, together with the classification of each constituent, were considered to be sufficient for the identification of individual constituents. The citronellyl acetate and geranyl acetate peaks were small and overlapped other peaks on both stationary phases so that this technique could not be applied to them. These peaks were identified by adding a little of the authentic specimens to the oil and noting the increase in size of two of the ester peaks.

The hydrocarbons were not identified, because they contribute little to the aroma of the oil.

PROCEDURE

GAS CHROMATOGRAPHY—

The gas chromatography was carried out on an argon ionisation instrument at a temperature of 100° C (except with diglycerol, which gave better results at 70° C) and a flow rate of 60 ml per minute. The support material was Celite (100 to 120 mesh) prepared from Celite 545 by wet-sifting. After it had been sifted it was heated to a temperature of 800° C overnight in order to reduce the adsorption of alcohols. All of the column packings contained 1 part by weight of stationary phase to ten parts of Celite. The packed length of all columns was 4 feet, and the diameter was 4 mm. The samples were injected by means of a glass capillary pipette containing approximately 0·05 μl.

SEPARATION OF HYDROCARBONS FROM OXYGENATED COMPOUNDS—

The hydrocarbons were separated from the oxygenated compounds by the method described by Kirchner and Miller.[5] Geranium oil (10 g) was added to 200 g of silica gel contained in a sintered-glass filter funnel, and the hydrocarbons were eluted by n-hexane. When this process was complete, ethyl acetate was passed through the silica gel until nothing further could be eluted.

The solvents were removed from the oil fraction by distillation through a Dufton fractionating column, and the residues were warmed under reduced pressure in order to remove the last traces of solvent.

HYDROLYSIS OF ESTERS—

The oil (2 ml) was heated under reflux for one hour with 20 ml of 0·5 N alcoholic potassium hydroxide. Distilled water (50 ml) was then added, and the products of the hydrolysis were isolated by extraction into ether.

ACETYLATION OF ALCOHOLS—

The oil (10 ml) was heated under a reflux air condenser with 20 ml of acetic anhydride and 2 g of freshly fused sodium acetate in accordance with the method described in British Standard 2073.[7] After 2 hours the mixture was cooled, 50 ml of distilled water were added, and the mixture was heated for 15 minutes on a boiling-water bath. The mixture was cooled, neutralised by adding 10 per cent. w/v sodium carbonate solution, and the acetylated oil was extracted with ether.

REMOVAL OF CARBONYL COMPOUNDS—

The oil (2 ml) was added to N hydroxyammonium chloride reagent containing dimethyl yellow indicator in a stoppered test-tube. The tube was placed in a water bath at about 80° C, and the liberated acid was neutralised at intervals of 5 minutes with N alcoholic potassium hydroxide until no further reaction occurred. The reaction mixture was then poured into water, and the precipitated oil was recovered by extraction with ether.

REMOVAL OF ALDEHYDES—

Dimedone (0·5 g) was dissolved in the minimum volume of alcohol at room temperature, and 1 ml of geranium oil was added. The mixture was set aside overnight, and was then poured into water. The oil was recovered by extraction with ether.

PREPARATION OF POLYETHYLENE GLYCOL SALICYLATE—

Polyethylene glycol 400 (20 g) and 14 g of salicylic acid were placed in a round-bottomed 250-ml flask together with 1 ml of concentrated sulphuric acid and 100 ml of n-heptane. The flask was fitted with a Dean and Stark apparatus and was heated on an electric heating mantle until the evolution of water ceased. This occurred when 1·6 ml had been collected. The mixture was then poured into 200 ml of water with vigorous stirring; three layers were formed. The heptane layer was separated, and the other layers were extracted with ether. The heptane layer and the ethereal extracts were each shaken with three portions of 5 N sodium hydroxide, and the extracts were then combined and acidified with dilute hydrochloric acid. An oil consisting of the desired ester and salicylic acid was precipitated. A small excess of ammonium hydroxide was added with vigorous stirring in order to dissolve the salicylic acid without dissolving the phenolic ester, which was then extracted with ether. Polyethylene glycol salicylate (10 g) was isolated as a viscous liquid.

RESULTS

The compounds listed below were identified—

Esters	*Ketones*	*Alcohols*
Citronellyl acetate	Isomenthone	Citronellol
Citronellyl formate	Menthone	Geraniol
Geranyl acetate		Linalol
Geranyl formate		Nerol

Twenty-two hydrocarbons (mainly in trace amounts), one aldehyde, two ethers and trace amounts of four esters were detected but not identified.

DISCUSSION OF RESULTS

Ideally, if all of the constituents of a mixture were completely separated, treatment with group reagents, such as hydroxyammonium chloride, would either remove a peak entirely or leave it untouched. Partial removal of a peak would indicate incomplete separation. In practice, however, this is not always true. The reaction may be incomplete. Acidic and alkaline reagents, for example, may cause the re-arrangement, decomposition or polymerisation of terpenoid compounds in addition to the desired esterification of alcohols or hydrolysis of esters.[8] Fortunately, however, the occurrence of this effect can often be detected. The instances when this occurred in the work described here are given below.

Although the compounds giving the group of small peaks at the beginning of the chromatogram (see Fig. 2) were partly removed by alcoholic potassium hydroxide solution, they were eluted from silica gel with hexane and appeared in the chromatogram of the hydrocarbon fraction.

The isomenthone peak also was decreased in height by this reagent, but was entirely removed by hydroxyammonium chloride. The alkali also caused a considerable increase in the height of the small peak containing menthone and a hydrocarbon. Acetylation reduced the sizes of the isomenthone peak and the largest hydrocarbon peak.

Acetylation removed the citronellol and geraniol peaks almost entirely, but a small residual peak that was not affected by repeating the acetylation procedure on the acetylated oil remained in place of each of these large peaks. The retention times of these peaks were determined and, within the limits of experimental error, were equal to those of the original large peaks. This occurred with both the polyethylene glycol column and with the salicylate column.

It is possible that the geraniol and citronellol peaks mask minor constituents and some peaks certainly overlap other small ones, but there is no evidence to show that peaks representing major components coincide with each other. In most instances of overlapping a "shoulder" can be detected in the peaks. Consequently, one may conclude that a 4-foot column containing 10 per cent. of polyethylene glycol 400 is able to separate completely all of the major constituents of geranium oil. The use of longer columns and higher detector sensitivites will no doubt reveal the presence of many more minor constituents, some of which may have an important influence upon the odour of the oil. It is evident, for example, that the group of terpenes at the beginning of the chromatogram is not fully resolved.

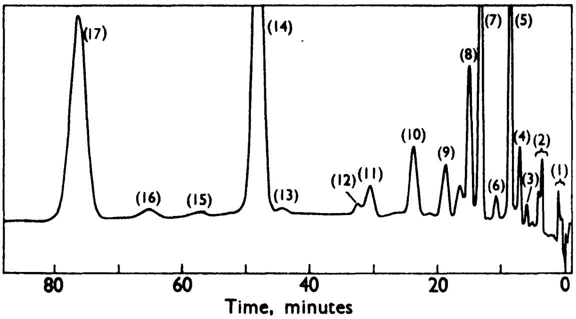

Fig. 11. Chromatogram of Algerian geranium oil with polyethylene glycol 400 as stationary phase. Peaks: (1) hydrocarbons; (2) ethers; (3) hydrocarbon; (4) hydrocarbon + menthone; (5) isomenthone; (6) hydrocarbon; (7) linalol; (8) citronellyl formate; (9) citronellyl acetate; (10) geranyl formate; (11) aldehyde; (12) geranyl acetate; (13) ester; (14) citronellol; (15) nerol; (16) ester; (17) geraniol

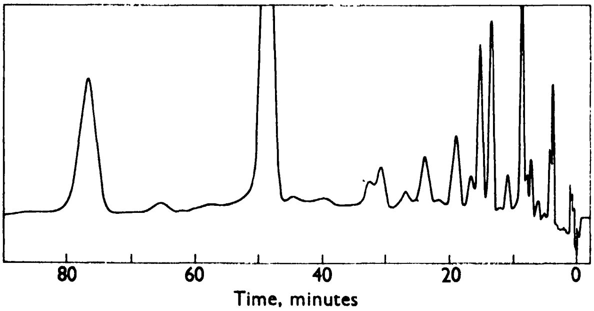

Fig. 12. Chromatogram of Moroccan geranium oil with polyethylene glycol 400 as stationary phase

Polyethylene glycol salicylate was found to be less selective than the glycol from which it was prepared. The procedure used for the identification of the peaks was identical with that used with the polyethylene glycol column.

It was found that geranyl formate was eluted together with citronellol, and the major hydrocarbon peak coincided with the citronellyl formate peak. This was indicated by the partial removal of the relevant peaks by group reagents and was confirmed by obtaining chromatograms of synthetic mixtures of these pairs of compounds. The results obtained on this stationary phase were consistent with those obtained by the use of polyethylene glycol 400.

This stationary phase was, however, able to separate cineole from the ethers present in the oil, whereas polyethylene glycol 400 could not separate it from the first of the two peaks produced by these substances.

Application of method

The analytical constants are of some assistance in assessing the quality of an oil, but it is known that an oil with satisfactory constants may have a poor odour, and it is impossible to determine the source of an oil by means of its constants alone.

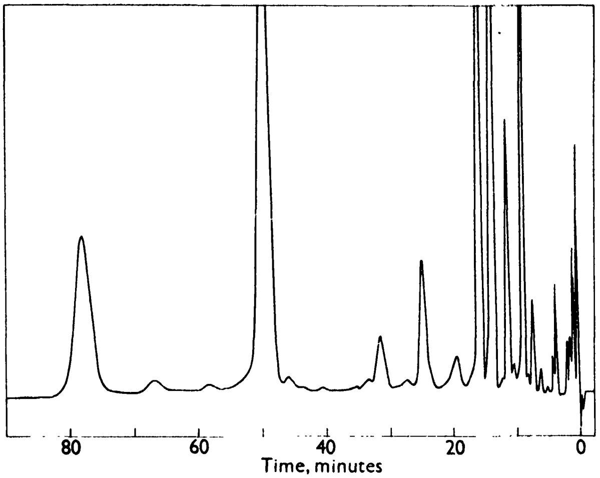

Fig. 13. Chromatogram of Réunion Bourbon geranium oil with polyethylene glycol 400 as stationary phase

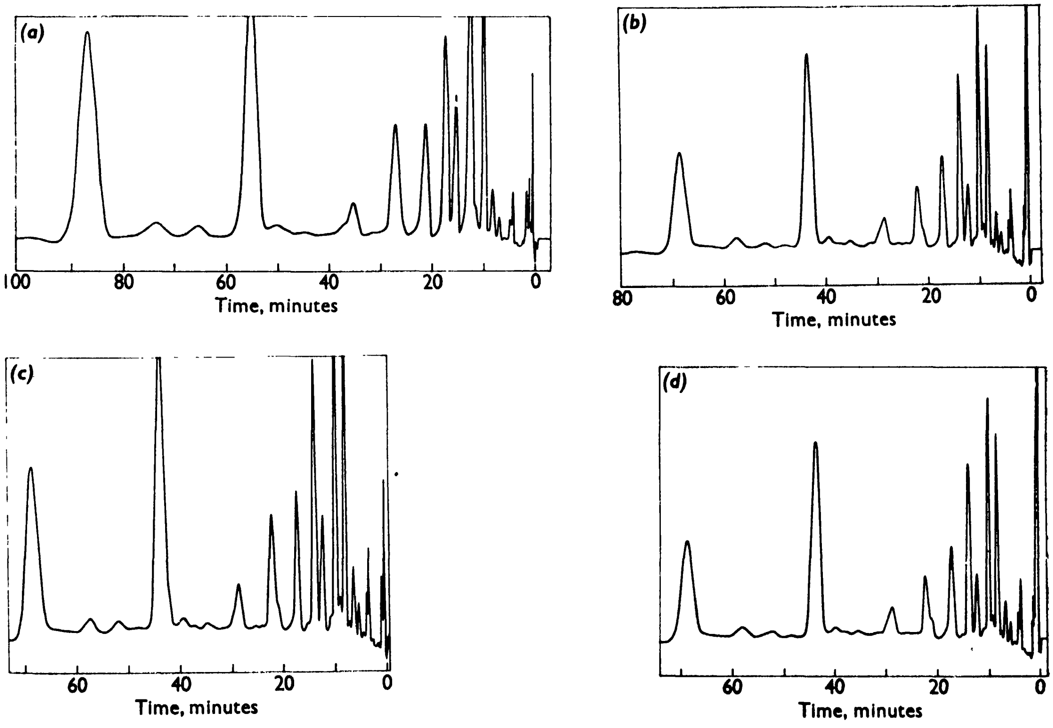

Fig. 14. Chromatograms of Kenya geranium oils taken from plants at different stages of maturity: (*a*) first harvest; (*b*) second harvest; (*c*) third harvest; (*d*) fourth harvest. Stationary phase, polyethylene glycol 400

A comparison of the chromatograms of specimens of geranium oils from Algeria, Morocco, Réunion and Kenya (see Figs. 11, 12, 13 and 2) shows that each one differs from the others in the relative heights of the peaks. Some differences of this kind are to be expected, even between good quality oils from the same source, but it can be seen that the patterns produced by Algerian and Moroccan oils share certain characteristics that differentiate them from the Réunion and Kenya oils, which also have much in common with each other. This is consistent with the accepted view that Algerian and Moroccan oils are of the same type, whereas Bourbon oils are of a different type and come, indeed, from a different variety of plant.

The most important difference exhibited by these two groups of oils is that the chromatogram peaks representing citronellyl formate and geranyl formate (which have a considerable influence on the odour of the oil) are relatively smaller for the North African oils. The hydrocarbon peak that emerges between isomenthone and linalol is also much smaller.

The aroma of Réunion oils is generally considered to be somewhat different from that of Kenya oils, in spite of the fact that they are derived from a common botanical source. A comparison of chromatograms of specimens of the two oils (see Figs. 13 and 2) supports this view.

The differences between these two oils must therefore be due either to differences in environment, in the maturity of the plants when harvested, in distillation technique or to a combination of these factors. In order to determine the effect of age of the plant upon the composition of the oil, the Scott Agricultural Laboratories of the Kenya Department of Agriculture carried out a series of small-scale harvesting trials in which four batches of plants were cut and distilled at various stages of maturity.

The oils thus produced were examined by me on a column containing 10 per cent. of polyethylene glycol 400, and the chromatograms are shown in Fig. 14.

If these chromatograms are compared with that of the specimen of Réunion oil (Fig. 13) it will be seen that all of the samples were rather deficient in linalol and contained rather more of the hydrocarbon and of citronellyl acetate than did the Réunion oil, but as the plants became more mature the proportion of citronellol increased considerably and the geraniol content decreased. The citronellyl formate and isomenthone peaks also increased in height. Thus the similarity between the Kenya oil and the Réunion oil increased with the maturity of the plants, though some differences persisted.

CONCLUSION

Polyethylene glycol 400 was found to give a more complete separation of the constituents of geranium oils than the other stationary phases tried. Although geranium oils contain traces of many compounds that cannot be detected without previous concentration by means of preparative-scale gas chromatography (D. Holness, personal communication), the chromatograms obtained by the method described here are sufficiently complete to provide useful information about the origin and quality of the oil.

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SHORT PAPERS

The Paper Chromatography of Some Substituted Cinnolines with Aqueous Solvents

Part II. With Aqueous Hydrochloric Acid and Sodium Hydroxide as Solvents

By R. J. T. GRAHAM*

(*Chemistry Department, Derby and District College of Technology, Kedleston Road, Derby*)

The chromatography of cinnolines in aqueous solutions of ammonium chloride and ammonium hydroxide has been reported.[1] Here the use of 0·1 N hydrochloric acid and 0·1 N sodium hydroxide is discussed.

The experimental conditions were identical with those previously reported.[1]

The R_F values, the mean of 5 runs, reproducible to ±0·02 R_F unit, are shown in Table I.

TABLE I

R_F VALUES FOR SUBSTITUTED CINNOLINES

Compound	R_F value in hydrochloric acid, 0·1 N	R_F value in sodium hydroxide, 0·1 N
Quaternised 4-*methylcinnolines*—		
1-Ethiodide-	0·00	C
6-Chloro-1-ethiodide-	0·00	C
7-Chloro-1-methiodide-	0·00	C
7-Chloro-1-ethiodide-	0·00	C
7-Carboxylic acid-1-ethiodide-	0·00	C
4-*Hydroxycinnolines*—		
7-Chloro-	C	0·33
6,7-Dichloro-	C	0·42
6-Chloro-7-methyl-	C	0·51
6-Bromo-7-methyl-	C	0·47
8-Methyl-6-nitro-	C	0·50
7-Methyl-6-nitro-	C	0·46
3-Ethyl-	C	0·61
7-Chloro-8-nitro-	C	0·45
3-Methyl-8-nitro-	C	0·42
7-Methyl-8-nitro-	C	0·54
4-*Methylcinnolines*—		
6-Chloro-	0·82	0·51
7-Chloro-	0·85	0·53
7-Carboxylic acid	0·78	0·68
7-Carboxylic acid ethyl ester	0·57	0·78

C = Comet-shaped spots unsuitable for measurements.

DISCUSSION

QUATERNISED 4-METHYLCINNOLINES—

These compounds remained at the point of application in acid solution and streaked in alkaline solution. This confirmed that streaking was caused by alkaline decomposition, and the separation of the decomposition products.

4-HYDROXYCINNOLINES—

These streaked in acid solution and migrated in alkaline solution, confirming that they migrate satisfactorily only when tautomerism is prevented.[1]

The R_F values in sodium hydroxide are similar to those reported for the ammonium hydroxide systems.[1]

---

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Separation of the isomeric pairs 8-methyl-6-nitro- and 7-methyl-6-nitro-, and 3-methyl-8-nitro- and 7-methyl-8-nitro is possible in this system, though the ΔR_F values are smaller than in the ammonium hydroxide systems. This system also resolves the 8-methyl-6-nitro- and the 7-methyl-8-nitro-compounds; the ammonium hydroxide system does not. In the ammonium hydroxide systems and in the sodium hydroxide system the presence of the methyl group in the hetero-ring gives lower R_F values than when it is in the benzene ring.

The 6-chloro-7-methyl- and the 6-bromo-7-methyl compounds were separable, though the ΔR_F values were smaller than for the ammonium hydroxide systems.

4-METHYLCINNOLINES—

These gave discrete spots in both acidic and basic solvents. Neither system resolved the isomeric 6-chloro- and 7-chloro-compounds, but the carboxylic acid and its ethyl ester were separable in both.

Previously, the R_F values in acid solution (5 per cent. w/v ammonium chloride solution) were lower than those obtained from basic solutions.[1] Here the values in acid solution are higher than the values in basic solution, probably because of the formation of stable hydrochlorides on the basic 1-nitrogen atom,[2,3] thus preventing the formation of the hydrogen bond between this atom and the cellulose surface.

I thank Dr. C. M. Atkinson for providing the cinnolines.

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NOTE—Reference 1 constitutes Part I of this series.

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Detection, Determination and Identification of Furfuraldehyde in Hydrocarbon Oil

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IT has been brought to our notice by Mr. C. H. R. Elston of Messrs. Williams (Hounslow) Ltd. that the determination of furfuraldehyde by the recently described modification of the aniline acetate method[1] is subject to variation with temperature, and also that improved repeatability can be obtained if the aniline acetate reagent is prepared with glacial acetic acid treated in the manner described by Fleury and Poirot.[2]

The reagents compared were prepared as described below.

Reagent I. Dissolve 100 ml of analytical-reagent grade aniline in 900 ml of glacial acetic acid. Store in a dark-coloured bottle.

Reagent II. Stand 1 litre of glacial acetic acid in contact with about 20 ml of aniline for 24 hours, and recover 90 per cent. by distillation. Dissolve 100 ml of analytical-reagent grade aniline in 900 ml of the distilled acetic acid. Store in a dark-coloured bottle.

To investigate the temperature effect, determinations at different temperatures were made of the furfuraldehyde contents of gas oils marked with 10·0, 5·0 and 1·0 p.p.m. of furfuraldehyde. The maximum optical density was found to vary with the temperature at which the entire experiment was carried out. The results with a particular aniline acetate reagent showed a variation from 0·810 at 15·4° C to 0·565 at 27·3° C for a concentration of 10·0 p.p.m. of furfuraldehyde, a decrease of 0·021 in the maximum optical density per 1° C rise in temperature, equivalent to 0·3 p.p.m. of furfuraldehyde per 1° C. The effect of temperature was much less at lower concentrations of furfuraldehyde. At 5·0 p.p.m. of furfuraldehyde the variation in maximum optical density with temperature was 0·003 per °C, equivalent to a decrease of 0·04 p.p.m. of furfuraldehyde per 1° C rise in temperature, and at 1·0 p.p.m. of furfuraldehyde the temperature effect was not detectable.

To compare the repeatability obtainable with the aniline acetate reagents prepared by the two methods described, a series of determinations was carried out at a fixed temperature on the gas oil containing 10·0 p.p.m. of furfuraldehyde by two operators using both reagents. The results in Table I show that there is no significant difference in repeatability by a single operator using either of the reagents. Table I also shows the important fact that the maximum optical density depends on the particular reagent used, Reagent II producing higher maximum optical densities than Reagent I.

The average maximum optical density given by 10·0 p.p.m. of furfuraldehyde with Reagent I, as calculated from many earlier determinations, is 0·737; the corresponding average for Reagent II, calculated from fewer determinations, is 0·798. Repeatability of 0·010 is equivalent to 0·14 p.p.m. of furfuraldehyde with Reagent I, and 0·13 p.p.m. of furfuraldehyde with Reagent II.

RECOMMENDED PROCEDURE—

The procedure described below for determining furfuraldehyde is standard practice in this Laboratory, and is recommended.

Carry out the determinations under such conditions that the samples (including the standards) and reagents before mixing are at a uniform temperature, and that while spectrophotometric readings are being taken the temperature of the solution in the cell does not vary by more than 1° C. Add the reagent to the gas oil, mix, and adjust the volume in the flask to the mark with toluene at this uniform temperature. Mix the contents of the flask, transfer a portion of the liquid to the cell of a spectrophotometer, and record the temperature of the liquid in the cell. Observe the optical density of the liquid at 520 mμ until the maximum optical density is reached, at which point record this figure, and take the temperature of the liquid in the cell to check that any change is within 1° C. For the most accurate work reject any result for which the temperature of the liquid in the spectrophotometer cell changed by more than 1° C. Make a determination on a standard solution of furfuraldehyde in the same manner at the same time and under the same temperature conditions as for the determination on the sample.

TABLE I

COMPARISON OF REPEATABILITY AT 23·0° C OF MAXIMUM OPTICAL DENSITY AT 520 mμ WITH REAGENTS I and II

	Optical density with Reagent I		Optical density with Reagent II		
	Operator 1	Operator 2	Operator 1	Operator 2*	
	0·742	0·750	0·822	0·795	0·808
	0·728	0·750	0·810	0·797	0·792
	0·740	0·747	0·817	0·794	0·797
	0·730	0·744	0·812	0·804	0·793
	0·738	0·756	0·808	0·804	0·801
	0·732	0·739	0·813	0·807	0·792
	0·721	0·738	0·810	0·794	0·790
	0·730	0·741	0·806	0·796	0·799
Mean ..	0·733	0·746	0·812	0·799	0·797
Maximum deviation from mean	−0·012	+0·010	+0·010	+0·008	+0·011

* The two columns relate to experiments with two separately prepared batches of Reagent II.

CONCLUSIONS—

It must be emphasised that accurate determinations of furfuraldehyde are only possible provided certain strict experimental conditions are observed.

The effect of temperature on the spectrophotometric determination of furfuraldehyde by reaction with aniline acetate has been confirmed. The maximum optical density is normally reached in a period of up to 4 minutes, and its value depends on the particular aniline acetate reagent employed and on the temperature of the experiment. It is recommended that freshly prepared reagent should be used. In our experience both Reagent I and Reagent II will give consistent and repeatable results, although it must be borne in mind that these results depend essentially on the particular reagent selected. If a laboratory is equipped with a constant temperature room, this naturally simplifies the entire experimental procedure. However, such a facility is rarely available.

In the system of exact analysis used in this Laboratory it is desirable to compare an unknown solution with a standard containing furfuraldehyde in an approximately equivalent amount. The concentration of this standard can easily be decided on by a preliminary test against a standard solution containing 10·0 p.p.m. of furfuraldehyde.

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A Glucose Oxidase Method for the Rapid Determination of Glucose in Starch Conversion Products

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PATTERSON and Savage[1] described a method for determining glucose and maltose in starch conversion products, based on the use of selective desorption from carbon columns. This method was later extended to determining maltotriose[2] and more recently to maltotetraose.[3] After separation of the sugars on charcoal, they are individually determined by the Somogyi method. For determining glucose alone in many hydrolysed starch samples, this carbon-column procedure with subsequent volumetric titration is time-consuming. The increasing use of glucose oxidase reagents for determining glucose in biological fluids[4,5] suggested that such an enzymatic method might be of use in the analysis of starch conversion products. Crowne and Mansford,[6] however, examined the effects of commercially available preparations of glucose oxidase on chromatographically pure maltose, maltotriose and maltotetraose, and showed that neither the "crude" nor the "pure" commercial preparations could be regarded as specific for glucose. During further studies on intestinal maltase, we found that Ellis's[7] universal buffer system, which makes use of 2-amino-2-methylpropane-1,3-diol, had a pronounced inhibitory effect on maltase. A glucose oxidase reagent made up with this buffer showed a marked reduction in reaction with the maltose, whereas the reaction with glucose was unimpaired. Following on the observation by Larner and Gillespie[8] that several related amines, including tri(hydroxymethyl)methylamine (Tris) inhibited maltase and 1,6-oligoglycosidase, White and Subers[9] and Dahlqvist[10] have recently published details of glucose oxidase reagents based on Tris and showing similar negligible maltase activity. We have now used this modified reagent for determining the glucose content of various commercially available starch conversion products.

EXPERIMENTAL

SOURCES OF ENZYMES—

Because results obtained earlier with the commercial "pure" glucose oxidase[6] showed that the maltase activity had also been enhanced by the purification, only commercial "crude" grades of glucose oxidase have been used. These all have a similar order of activity of about 1100 units per μg.[11] The crude grades of glucose oxidase were obtained from L. Light and Co. and the British Drug Houses Ltd. The horseradish peroxidase was obtained from C. F. Boehringer und Soehne.

Anhydrous α-D-glucose of analytical-reagent grade was used. Pure maltose was prepared by column chromatography on charcoal of the commercial maltose (containing glucose and maltotriose). Maltotriose and maltotetraose were prepared as described earlier.[12] Normal Liquid Glucose DE.42 and high conversion Liquid Glucose DE.63 were donated by Garton, Sons and Co. Ltd., "Morsweet" by Brown and Polson Ltd. and "Starcose" by Starch Conversions Ltd. of Wigan.

ENZYME - DYE REAGENTS—

Powdered "crude" glucose oxidase (1·25 g), 5 mg of horseradish peroxidase and 50 mg of recrystallised *o*-dianisidine were made up to 500 ml with the appropriate buffer solution to pH 7. After filtration, the reagent was stored in amber-glass bottles at 4° C.

The buffer solutions (pH 7) used were—

(*a*) ***Phosphate:*** 350 ml of potassium dihydrogen orthophosphate solution (27·23 g per litre) and 208 ml of 0·2 N sodium hydroxide.

(*b*) *Tris:* 30·5 g of tri(hydroxymethyl)methylamine in 42·5 ml of 5 N hydrochloric acid made up to 500 ml.

ENZYMATIC DETERMINATION OF GLUCOSE—

The test solution (1 ml) usually containing between 50 and 200 mg of glucose per 100 ml was incubated with 10 ml of the appropriate enzyme - dye reagent for 45 minutes at 37° C. After the mixture had been cooled, the intensity of colour produced was measured at 450 mμ in 1-cm glass cells with a Unicam SP600 spectrophotometer. Standard solutions of glucose containing 50, 100, 150 and 200 mg per 100 ml were run at the same time as the test solutions.

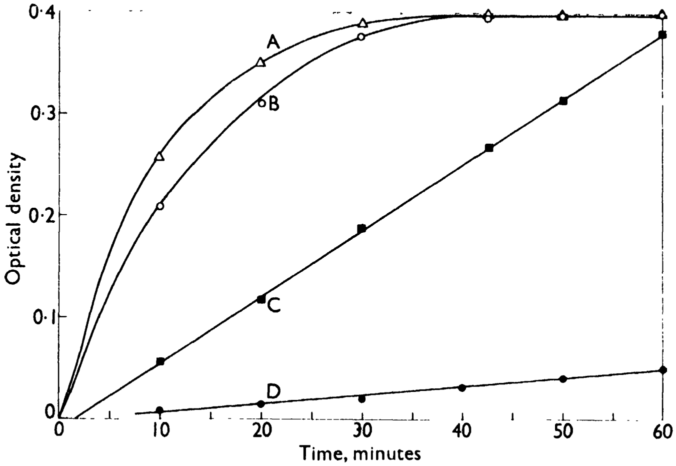

Fig. 1. Effect of buffer composition of glucose reagent on the development of colour: curve A, 50 μg of dextrose (phosphate buffer); curve B, 50 μg of dextrose (Tris buffer); curve C, 2 mg of maltose (phosphate buffer); curve D, 2 mg of maltose (Tris buffer)

Initially, the inhibitory action of Tris on the maltase activity of commercial glucose oxidase preparations was confirmed (see Fig. 1). A plot of optical density against time showed that, over the incubation period of 45 minutes, the normal phosphate buffer reagent produced considerable reaction with maltose. When the phosphate buffer was replaced by Tris the optical density obtained from the glucose was unchanged after 45 minutes' incubation, whereas with maltose the optical density was reduced to an insignificant level.

TABLE I

DETERMINATION OF GLUCOSE IN COMMERCIAL STARCH CONVERSION PRODUCTS

Sample	Glucose found by carbon column method, % w/v	Glucose found by enzymatic method (high level), % w/v	(low level), % w/v
Liquid Glucose DE.42	16·4	16·5	16·6
Liquid Glucose DE.63	30·2	30·4	30·3
Morsweet (high maltose)	5·8	5·9	5·9
Starcose	4·6	4·8	4·7

The effect of Tris buffer reagent was then determined on standard amounts of chromatographically pure maltose, maltotriose and maltotetraose (see Fig. 2) and here again there was a striking reduction in the optical density observed. As a further check on the efficacy of the

Tris buffer reagent as an inhibitor of the maltase, a set of standard solutions of glucose in water (50 to 200 mg per 100 ml) was prepared and analysed, and the results were compared with those from a similar set of glucose standards made up in a solution containing 200 mg of maltose per 100 ml; identical standard curves were obtained for both series of solutions.

Finally, four different commercial starch conversion products were analysed by the carbon column method[1] to obtain their percentage glucose contents. These materials were then analysed by the glucose oxidase - Tris buffer reagent method at two concentration levels; the results are compared in Table I.

DISCUSSION

Whistler, Hough and Hylin[13] removed amylase and maltase activities from commercial glucose oxidase, but with only a 20 per cent. recovery of glucose oxidase activity. Both Dahlqvist[14] and Friedman,[15] however, were unable to remove maltase activity. Friedman[15] noted that Tris was inhibitory to trehalase and maltase contaminants in commercial glucose oxidase, but did not apply this observation analytically. Both White and Subers[9] and Dahlqvist[10] used Tris-buffered glucose oxidase reagent for the assay of maltase activity. We have found this modified glucose oxidase reagent to be both reliable and rapid in use. When many starch conversion products are to be assayed solely for glucose content, it has the advantages of simplicity and speed over the carbon column method.

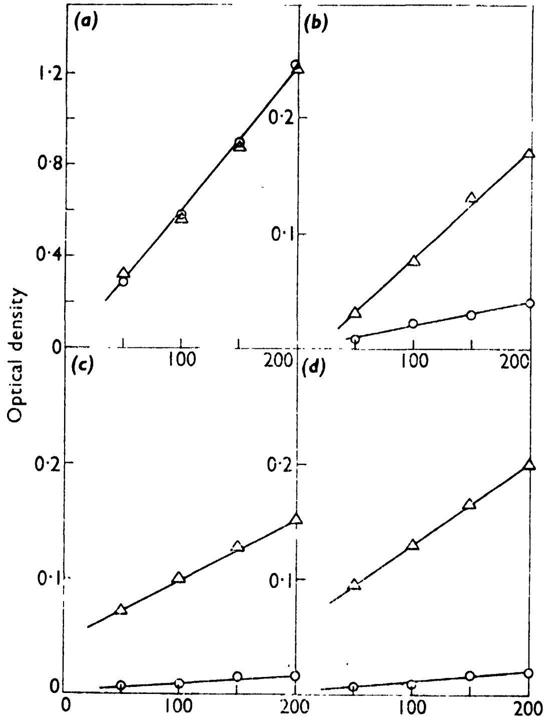

Fig. 2. Effect of buffer composition of glucose oxidase reagent on the reaction with: (**a**) dextrose; (**b**) maltose; (**c**) maltotriose; (**d**) maltotetraose. △, phosphate buffer; ○, Tris buffer

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The Determination of Maltotetraose in Starch Conversion Products

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LIQUID glucose has been a standard raw material in the food and confectionery industry for over 50 years and has been available in a limited number of grades of different degrees of conversion of the starch. Of recent years there has been a demand for a wider variety of conversion products. This has led to the use of enzymes for industrial processing and a consequent need for carbohydrate analysis in greater detail than the scale of "dextrose equivalent" used hitherto.

Patterson and Savage[1] provided the basis for the detailed analysis of starch conversion products by their use of selective desorption from carbon columns for the determination of dextrose and maltose. This method was later extended to the determination of maltotriose.[2] Conditions have now been worked out for the quantitative separation of maltotetraose from starch conversion products, and details are given here of the application of this method to various commercial glucose syrups.

EXPERIMENTAL

Seven grams of charcoal - Celite mixture (2 + 1) were mixed to a slurry with boiled water and packed into a jacketed glass column maintained at 40° C. After a brief wash with water, 5 ml of a 2 per cent. w/v solution of the starch conversion product were applied to the top of the column. The sugars were then eluted by stepwise increases in the concentration of ethanol, as indicated below—

Dextrose	100 ml of water
Maltose	100 ml of 5 per cent. aqueous ethanol
Maltotriose	150 ml of 8 per cent. aqueous ethanol
Maltotetraose	200 ml of 12 per cent. aqueous ethanol

Determination of the individual sugars was then carried out by the Somogyi method as described by Patterson and Savage.[1]

Standardisation of the Somogyi reagent for maltotetraose was carried out on pure maltotetraose obtained by preparative-scale charcoal column chromatography of liquid glucose with 12 per cent. aqueous ethanol as described earlier.[3] The heating time was maintained initially at 20 minutes. The standardisation values against 0·005 N sodium thiosulphate are shown below since the difficulty in obtaining pure maltotetraose may prevent individual standardisation in other laboratories. The standardisation values have been confirmed by Lees[4]; they are—

Maltotetraose (20 minutes heating), mg ..	0·1	0·2	0·4	0·6	0·8	1·0
0·005 N sodium thiosulphate, ml	0·15	0·32	0·64	0·97	1·28	1·62

PAPER CHROMATOGRAPHY—

During the investigations on the optimum conditions for separating maltotetraose, the column was run in conjunction with a fraction collector of the siphon-balance type, and 20-ml fractions were collected throughout the separation. Two-millilitre portions of each of these fractions were evaporated to dryness *in vacuo* and examined by paper chromatography. Frame chromatography tanks, as described by Datta, Dent and Harris,[5] were used at a temperature of 50° C in a thermostatically controlled oven. Under these conditions an ascending solvent run of 2½ hours with Whatman No. 1 paper in n-butanol - ethanol - water (2 : 1 : 1) gave excellent separations of all the saccharides up to maltopentaose. The sugars were revealed by reaction with aniline - diphenylamine reagent.[6]

SIZE OF COLUMNS—

The dimensions of the column used (18 inches × 0·5 inch) were essentially similar to those used in the longer, narrower columns described by Patterson and Buchan.[2] The short columns (6 inches × 0·75 inch) used in the determination of dextrose and maltose were found to give tailing of the maltotriose into the maltotetraose.

ADSORBENT—

Patterson and Savage[1] described a test for the suitability of a charcoal for use in the analysis of starch conversion products and found only one commercially available carbon capable of giving quantitative recovery of sugars when used as supplied. Although their preferred charcoal (B.D.H. Activated Charcoal, Acid Washed) was found to be of value, some difficulty was experienced on occasions in obtaining consistent results with this material. In general, it was found that Active Carbon No. 130 (Sutcliffe, Speakman & Co. Ltd., Widnes, Lancs.) gave a higher capacity and consistent adsorptive behaviour. Stearic acid de-activation of the charcoal as described by Alm[7] and used by Miller, Dean and Blum[8] was not found to have any beneficial effect on the separation achieved. Active Carbon No. 130 satisfies the acidity criterion recently described by Taylor and Whelan.[9]

The charcoal - kiesulguhr mixture (1 + 1) used by Patterson and Savage[1] was replaced by a mixture of charcoal - Celite 545 (2 + 1). It was found that Celite 545 provided faster flow rates than did kiesulguhr and allowed a higher proportion of charcoal to be incorporated in the 7 g of adsorbent used in the column. With both the B.D.H. and the Sutcliffe Speakman grades of charcoal this mixture then provided a clean separation of the maltotetraose from the maltopentaose without the overlap recently experienced by Lees.[4]

EFFECT OF TEMPERATURE AND ETHANOL CONCENTRATION ON SEPARATION—

The volume of aqueous ethanol required to elute the maltose quantitatively from a carbon column is considerably influenced by temperature, although the volume of water required to elute the dextrose is not affected to the same degree. When 7 per cent. aqueous ethanol was used, as suggested originally by Patterson and Savage,[1] it was found that the maltose was eluted readily at room temperature, but the maltotriose tended to follow the maltose peak immediately, so that some overlap of the two components could occur. Reducing the ethanol concentration to 5 per cent. removed this difficulty and all the maltose was eluted in 100 ml.[2] At this stage the ethanol concentration was raised to 7 per cent. Maltotriose was then eluted as a diffuse band with a pronounced tail. Increasing the temperature of elution first to 30° C and then to 40° C reduced the tailing, but an excessive volume of 7 per cent. ethanol was still required to elute all the maltotriose. Increasing the ethanol concentration to 8 per cent. and eluting at 40° C, however, provided a much sharper maltotriose peak, and all the maltotriose was eluted in 150 ml. Maltotetraose could then be eluted with 10 per cent. aqueous ethanol, again at 40° C. However, some 300 ml of this 10 per cent. ethanol was required to elute all the maltotetraose. By using 12 per cent. aqueous ethanol at 40° C it was found possible to elute all the maltotetraose in 200 ml without any interference from maltopentaose. Further elution of the column with 60 per cent. aqueous ethanol was carried out, and the solution was evaporated to dryness *in vacuo* before paper chromatography. The chromatogram revealed no maltotetraose in this 60 per cent. ethanol fraction.

RECOVERY OF MALTOTETRAOSE—

The recovery of maltotetraose from the charcoal column by elution with 12 per cent. aqueous ethanol at 40° C was checked with 10 mg of pure maltotetraose.[3] The recovery in a series of three experiments varied between 98 and 104 per cent.

ANALYSIS OF COMMERCIAL STARCH CONVERSION PRODUCTS

A histogram of a typical separation of dextrose, maltose, maltotriose and maltotetraose is shown in Fig. 1.

TABLE I

RESULTS

Starch conversion product	Dextrose found, %	Maltose found, %	Maltotriose found, %	Maltotetraose found, %
Liquid Glucose DE.42 ..	16·4	11·1	9·9	9·0
Liquid Glucose DE.63 ..	30·6	22·4	9·5	7·2
Starcose	4·4	38·4	8·4	3·6

The method has been applied to several commercial starch conversion products produced by both acid hydrolysis and enzymatic hydrolysis. The results are summarised in Table I.

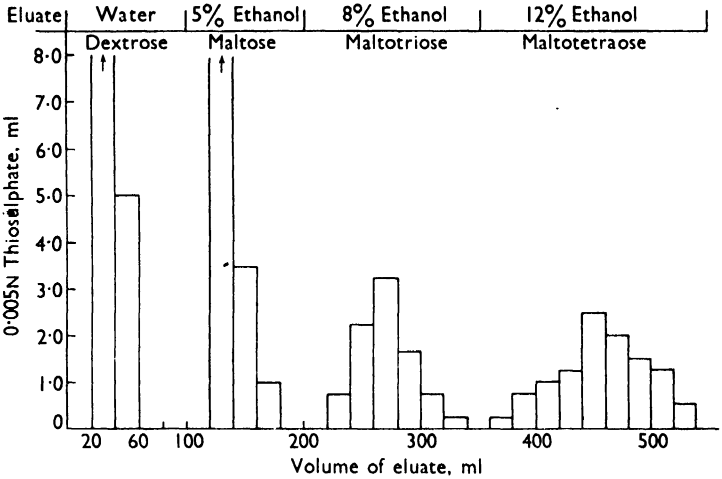

Fig. 1. Histogram of the selective desorption of dextrose, maltose, maltotriose and maltotetraose from charcoal - Celite columns

ISO-SUGARS—

For the enzymatic hydrolysed products the presence of iso-sugars (*e.g.*, iso-maltose, iso-maltotriose and panose) can easily be demonstrated chromatographically.[3] In the examples described here any 1,6 linked saccharides have been eluted from the column with the appropriate 1,4 linked saccharide fraction, so that no overlapping of the di- and trisaccharide fractions results from the presence of these iso-sugars. It has, however, been demonstrated with a large preparative-scale column that some degree of separation can be achieved of 1,4 disaccharide (maltose) from 1,6 disaccharides.[3] The error involved is negligible at the levels encountered to date, but the possibility of iso-sugar interfering with the separation and with the Somogyi determination should be considered when dealing with enzymatic starch conversion products.

I acknowledge with gratitude the skilled technical assistance of R. K. Opie and A. Wallace.

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The Determination of Dinoseb in Potatoes

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THE use of dinoseb (2-s-butyl-4,6-dinitrophenol) as a potato haulm killer has resulted in the need for a rapid method for determining traces of dinoseb in potato tubers.

From consideration of the methods used for the macro determination of dinoseb, both alone and in mixed weedkiller formulations,[1,2,3] it seemed that a suitable method for determining trace amounts could be devised, based on measurement of the intense yellow colour of the sodium salt.

On attempting to recover 50- to 100-μg amounts of dinoseb from 100 g of potato by extraction with dilute aqueous sodium hydroxide or sodium carbonate, considerable interference was encountered. Maceration of the potato with dilute sodium carbonate solution, and then acidification of the filtrate, extraction with ether and re-extraction into sodium carbonate solution for measurement of the colour gave only about 30 per cent. recovery of the added dinoseb.

Dinoseb itself is readily steam volatile, but experiments showed that it could not be separated satisfactorily from potato by steam-distillation.

An attempt was then made to adapt Fenwick and Parker's method for determining 3,5-dinitro-*o*-cresol (DNOC) in biological tissues,[1] in which the sample is macerated with chloroform in the presence of trichloroacetic acid and then extracted with dilute sodium carbonate solution, re-extracted into ethyl methyl ketone in the presence of sodium chloride, and the optical density of the yellow colour is measured. It was found that the principle of this method could be applied successfully to the determination of dinoseb.

The visible absorption spectrum of the sodium salt of dinoseb in ethyl methyl ketone was found to exhibit maxima at 379 and 429 mμ, thus affording a useful distinction from other possible phenolic substances, though not from DNOC, which has maxima at 377 to 430 mμ.

Dinoseb added to potato, in amounts of 0·5 p.p.m., 0·25 p.p.m. and 0·1 p.p.m. on a 100-g sample, was recovered to the extent of 90 $\pm$ 5 per cent., and zero blank values were obtained. The method has also been used successfully for determining dinoseb in potato haulm and in fodder crops.

METHOD

APPARATUS—

Spectrophotometer—Unicam SP500.
Homogeniser—*e.g.*, Ato-Mix 800 M.S.E.

REAGENTS—

Chloroform.
Ethyl methyl ketone.
Sodium carbonate solution, 2 per cent. w/v, aqueous.
Sodium chloride.
Sodium sulphate, anhydrous.
Trichloroacetic acid.

PROCEDURE—

Weigh 100 g of potato, obtained by subsampling, and macerate for 5 minutes in an Ato-Mix 800 or similar homogeniser, with 100 ml of chloroform and 5 g of trichloroacetic acid. Filter with suction through a Whatman No. 31 filter-paper, and wash the residue with three 20-ml portions of chloroform. Transfer the filtrate to a separating funnel. Run the chloroform layer through anhydrous sodium sulphate, supported by a cotton-wool plug, into a 100-ml extraction flask. Evaporate the chloroform on a water bath. Dissolve the residue in 5 ml of chloroform, add 10 ml of sodium carbonate solution, stopper the flask, and shake vigorously for 1 minute. Set aside for 15 minutes.

By pipette transfer a suitable aliquot, *e.g.*, 5 ml, of the clear aqueous layer to a small separating funnel, add about 1 g of sodium chloride, 5 ml of sodium carbonate solution and 5 ml of ethyl methyl ketone, and shake well for 1 minute. If an aliquot of the sodium carbonate extract other than 5 ml has been taken, add to the contents of the separating funnel sufficient sodium carbonate solution to bring the total volume to 10 ml.

Run off the ketone layer into a 10-ml cylinder, washing in with a few drops of ketone. Adjust the volume to 5 ml, filter into 1-cm cell, and measure the optical density of the solution at 379 mμ.

Interference due to non-phenolic plant pigments present in the ketone extract is corrected for by subtracting the optical density at 379 mμ after the addition of a drop of concentrated hydrochloric acid.

Calculate the dinoseb content of the sample by using a calibration curve obtained from standard dinoseb solutions.

The sensitivity of the method is indicated by the results shown below, obtained on technical dinoseb—

Weight of technical dinoseb, μg ..	10	25	50	70
Optical density	0·112	0·275	0·550	0·770

I thank the Atlas Preservative Co. Ltd. for the sample of technical dinoseb.

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The Determination of Antimony in Titanium Dioxide

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THE methods available for determining small proportions of antimony in titanium dioxide pigments (*e.g.*, <200 p.p.m. in anatase) are, in our experience, unsatisfactory. The method based on the production of a colour with potassium iodide and pyridine[1] gives a proportion of erratic results, and methods depending on a preliminary separation of the antimony as stibine[2,3] suffer from losses due to the inherent instability of the gas on glass and similar surfaces.[4]

We applied the recently published method of Stanton and McDonald,[5] after dissolving the titanium dioxide in sulphuric acid, and, by using the conditions described below, obtained results that showed satisfactory reproducibility and accuracy.

METHOD

REAGENTS—

All reagents should be of analytical grade whenever available.

Brilliant Green (*C.I. No.* 42040) *solution*, 0·05 *per cent. w/v, aqueous.* This must be freshly prepared before use.

Hydrochloric acid, sp.gr. 1·18.

Sodium chloride.

Sodium nitrite solution, 5 *per cent. w/v, aqueous.*

Sodium hexametaphosphate solution, 10 *per cent. w/v, aqueous.*

Sulphuric acid, sp.gr. 1·84.

Toluene.

Standard antimony solution—Dissolve 0·267 g of antimony potassium tartrate in sufficient water to produce 1000 ml. Dilute 2 ml to 100 ml with water.

1 ml 2 µg of antimony.

PREPARATION OF CALIBRATION GRAPH—

Transfer portions of standard antimony solution in the range 0 to 15 µg of antimony to a series of 50-ml stoppered flasks containing 8·2 ml of hydrochloric acid. Dilute to approximately 15 ml with water, add 0·5 ml of sodium nitrite solution, and mix; the oxidation should be carried out below 25° C. Immediately after the oxidation stage, add 25 ml of sodium hexametaphosphate solution and 5 ml of Brilliant Green solution, mixing after each addition. Extract with 5 ml of toluene, and measure the optical density against a reagent blank solution in a 1-cm cell at 640 mµ.

TREATMENT OF ANATASE SAMPLES—

Dissolve 0·8 g of sample in 30 ml of concentrated sulphuric acid by heating slowly until the solution just begins to become turbid. (Certain samples may be found to dissolve more readily in a mixture of 30 ml of sulphuric acid and 8 g of ammonium sulphate.) Cool, and dilute with water to about 90 ml; cool again, transfer to a 100-ml calibrated flask, and dilute to the mark with water. By pipette transfer 5 ml to a 50-ml stoppered flask, add 3 g of sodium chloride and 3·3 ml of concentrated hydrochloric acid, dilute to 15 ml with water, and then proceed exactly as described for the preparation of the calibration graph.

DISCUSSION OF RESULTS

Four calibration experiments were conducted on solutions containing 0 to 15 µg of antimony in the presence of 24 mg of titanium, and the graphs produced were rectilinear and congruent.

Experiments involving the addition of known amounts of antimony (in the range 0 to 200 p.p.m.) to titanium dioxide samples, before dissolution in sulphuric acid, gave recoveries between 96 and 98 per cent. Some skill is necessary to produce almost clear solutions of the samples, and our experiments demonstrated that unusually poor solutions were invariably associated with abnormally low results. Low results were also obtained if the solutions of the samples were allowed to stand for long periods before the analyses were completed.

The samples of anatase examined proved to contain between 50 and 500 p.p.m. of antimony.

We thank the Directors of Hopkin & Williams Ltd. for permission to publish this paper.

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An Improved Type of Weight Burette for use in Volumetric Analysis

By H. N. REDMAN

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Various types of weight burettes or pipettes have been described from time to time. Two of the best known are Ripper's burette and the Lunge-Rey pipette. An improved type of weight burette has been in use in this laboratory for about 5 years, and L. S. Theobald, in his review of the latest edition of "Chemical Analysis—The Working Tools" in the Journal of the Royal Institute of Chemistry for February, 1963, directed our attention to the fact that this burette has not yet been described in the literature; it is intended to remedy this omission here.

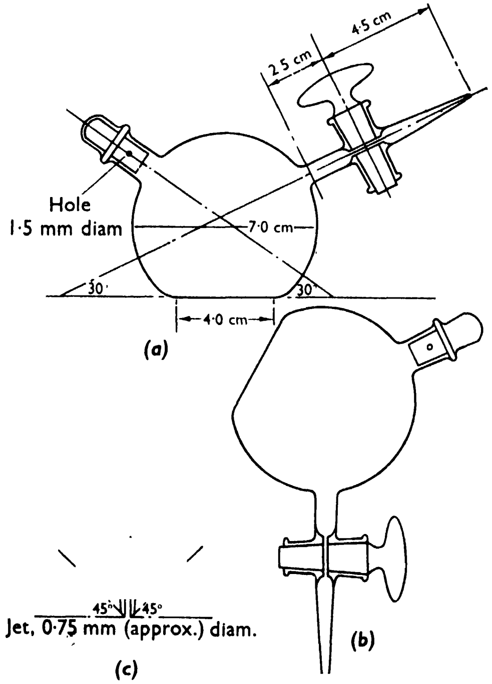

Fig. 1. Improved weight burette

The burette is shown in Fig. 1. It is essentially a flat-bottomed flask of capacity about 120 to 130 ml, fitted with a filling neck and a discharge jet. The filling neck is closed by a glass stopper, both the neck and stopper having 1·5-mm diameter holes drilled in them so that, by rotation of the stopper, air may be admitted to the burette. The discharge jet is closed by a small glass tap. The empty burette weighs between 50 and 60 g. The jet is shown diagrammatically at (*c*) in Fig. 1, and should have a gradual smooth taper. The end of the jet should be ground at an angle of 45° and then fire-polished. All surfaces of the apparatus are then silicone treated as described below. The burette (including tap and stopper) is thoroughly de-greased by immersion for about 15 minutes in a 10 per cent. solution of potassium hydroxide in industrial methylated spirit (methanol is unsuitable). After the burette has been washed with distilled water and dried in a stream of warm air, it is immersed in a 2 to 3 per cent. solution of silicone fluid in carbon tetrachloride. The excess of solution is allowed to drain from the surface, and the burette is heated at 110° C for 2 hours. When the apparatus is cool, the silicone treatment is repeated. The burette is then ready for use after the tap and stopper have been lightly greased. This procedure makes much easier the cleaning of the apparatus after use, and also causes extremely small droplets to form on the bore of the jet rather than on the outside circumference, which allows close control of volume during the titration.

The burette is placed in the balance case and allowed to come to equilibrium; the stopper is turned to equalise pressures, then closed again, and the burette is weighed. A suitable amount of the liquid is then introduced, allowed to come to equilibrium, pressures are equalised, and the burette is re-weighed. The design allows the burette to sit on the balance pan (see Fig. 1*a*) without danger of leakage or evaporation. For use in titration (see Fig. 1*b*) the burette is supported in a ring of suitable size covered with rubber tubing clamped at a convenient height on a retort stand. The stopper is then turned so that the holes coincide, and the burette is used in the usual way. After titration, the liquid remaining in the jet is allowed to run back into the bulb; this takes place completely because of the silicone treatment. The stopper is then turned to the closed position, thereby preventing evaporation. After equilibrium has been reached, the stopper is opened momentarily, and then the burette is re-weighed. On occasions when it is found necessary to leave the burette for some hours between weighings, during which time considerable changes in atmospheric conditions may occur, the method of weighing with a similar burette as counterpoise can be used to advantage. This is not possible with a constant-load single-pan aperiodic balance, and fortunately is not often necessary.

This burette is robust and convenient to handle, and can be recommended with confidence. The only precaution that might be mentioned is to handle the burette carefully, because the silicone treatment tends to make it slippery.

Received *March 15th*, 1963

Polythene Wash Bottles for Volatile Solvents

By V. H. BOOTH

(Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council)

In warm weather the more volatile solvents, such as diethyl ether, are liable to be spontaneously ejected from polythene squeeze bottles as a result of evaporative pressure. This leakage is easily prevented by puncturing the bottle's shoulder with a hot pin. The best position for the hole is soon found by manipulating an empty bottle. A thumb or finger is placed over the hole when the bottle is used. A circle drawn round the hole, for example, in ink, aids location.

Received *December 17th*, 1962

Book Reviews

QUALITATIVE ANION-CATION ANALYSIS: AN INTERPRETATIVE LABORATORY TEXT OF SEMIMICRO PROCEDURE IN BASIC COLLEGE CHEMISTRY. By EMIL J. MARGOLIS. Pp. x + 300. New York and London: John Wiley & Sons Inc. 1962. Price 38s.

This is a laboratory manual for beginners in qualitative analysis on the semi-micro scale. It deals with the 24 "common" cations and about 20 familiar anions, and provides a step-by-step course of instruction. The working directions are printed on alternate, coloured, pages and are faced by accounts of the chemistry involved, an arrangement that makes it easy for the student to follow the plan of the book, but which may restrict flexibility in modifying the course.

The sulphide group is precipitated by hydrolysis of thioacetamide, and the advantages and limitations of this reagent are clearly stated. Although the detection of fluoride, phosphate and oxalate is included, these anions are not, at this stage of instruction, expected to occur in mixtures in which they might interfere with cation analysis.

The manipulations of semi-micro work are described in a glossary, to which the student is referred when these manipulations are first required.

Teachers will find a great deal of information in the book, but they may consider the approach rather detailed for class-work at the early level at which elementary qualitative analysis is customarily taught in this country.

D. W. WILSON

THEORY AND APPLICATIONS OF ULTRAVIOLET SPECTROSCOPY. By H. H. JAFFÉ and MILTON ORCHIN. Pp. xvi + 624. New York and London: John Wiley and Sons Inc. 1962. Price 113s.

The authors are professors of chemistry at the University of Cincinnati and between them they have in recent years been responsible for independent lecture courses at the post-graduate level on molecular spectra and on applications of ultraviolet spectrophotometry. The first course was taken mainly by students "majoring in the physical and theoretical chemistry program" and the second "attracted primarily graduate students in the organic chemistry program."

Resonance theory has been very useful to organic chemists for correlating miscellaneous facts without using much mathematics, but although it lends itself to describing ground states it is less satisfactory than molecular-orbital theory for describing excited states. To help the reader to understand electronic absorption spectra the authors aim at a molecular-orbital framework of theoretical concepts "that would neither offend the molecular spectroscopist nor appear incomprehensively complex to the . . . organic chemists." "The principal device for effecting the necessary compromise between mathematical theory and qualitative development is the use of fine-print sections." In fact the use of a slightly smaller fount is not so much a compromise as a reflexion of the fact that the two elements of the book are imperfectly fused (perhaps necessarily).

There are clear accounts of atomic orbitals and the elements of molecular-orbital theory are expounded with respect to diatomic and a few relatively simple polyatomic molecules. After a theoretical discussion of symmetry operations, electronic states and intensities of absorption are dealt with as problems in molecular spectra. The transition to empirical absorption spectrophotometry is effected in a chapter on simple chromophores and solvent effects. This reads very well. It is followed by an orthodox treatment of conjugated dienes and $\alpha\beta$-unsaturated carbonyl compounds. Polyenes are discussed in terms of the free-electron model. The spectra of benzene and its derivatives and of polycyclic hydrocarbons are ably summarised, and there is a long chapter on the spectra of heterocyclic compounds, which although far from comprehensive is very instructive. An interesting account of steric effects completes this section of the book.

A short chapter on organic ions and free radicals is followed by a substantial account of the spectra of sulphur and phosphorus compounds. The spectra of various types of inorganic complexes are discussed in terms of the ligand-field theory. Fluorescence and phosphorescence spectra are dealt with from a largely theoretical standpoint. The final chapter, dealing with "application of spectra to quantitative analysis and the determination of equilibrium constants" is admirable.

There are three appendixes, one headed "Glossary and definitions; spectral notation; conventions."

The two authors belong to different camps. If the reader's main interest is in the rigorous treatment of molecular spectra he may feel disappointed that triatomic and tetratomic molecules have not been discussed more fully. On the other hand, readers who are mainly interested in organic molecules will skip a great deal of mathematical treatment. It cannot be doubted that the special qualities of the book will help to bridge a gap. There are perhaps too many reproductions of already accessible spectra of aromatic hydrocarbons, and the absence of reference to quinones is a pity.

This is, however, a well written book that deserves to be made available wherever chemistry is studied seriously.

R. A. MORTON

KOLORIMETRIE, PHOTOMETRIE UND SPEKTROMETRIE: EINE ANLEITUNG ZUR AUSFÜHRUNG VON ABSORPTIONS-, EMISSIONS-, FLUORESCENZ-, STREUUNGS-, TRÜBUNGS- UND REFLEXIONSMESSUNGEN. By GUSTAV KORTÜM. Pp. viii + 464. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1962. Price DM 48.

This fourth edition of a work that first appeared some 20 years ago deals mainly with absorption spectrophotometry, although there is some account of the observation of Raman and fluorescence spectra. The sub-title claims the inclusion of the measurement of scattering and turbidity, but in fact little space is devoted to these aspects, and the considerable post-war work on polymer molecules is hardly touched on.

The attention given to visual and photographic methods of photometry is quite properly much reduced in this edition, though the various forms of Duboscq colorimeter are still included. The section on applications has been entirely deleted. All this is to make room for a detailed consideration (150 pages) of photo-electric instruments for the visible and ultraviolet regions. In contrast only 40 pages are devoted to infrared spectrometry, and there is no mention of the relatively new interferogram methods so useful for the far infrared.

Many commercial spectrophotometers are described—German, British and American—but sometimes the name or manufacturer of an instrument is not to be found in the index. Apart from this feature, the subject index (12 pages in double column) is good. There is no author index and the considerable number of literature references is distributed at the bottoms of the pages.

The first 200 pages are devoted to an exposition of the principles of the subject (the nature of absorption, chromophoric groups, the laws of absorption, etc.) as well as to the principles used in spectrophotometers (lens systems, prism arrangements, photometric devices, radiation sources, detectors—photo-transistors are included—amplifying arrangements, specimen cells, solvents, etc.).

This is a very comprehensive work—naturally not everything can be included—which, with its extensive bibliography, will form a useful reference book. In particular, it will be useful to the British reader, who may not be so conversant with European as with British and American instruments. G. F. LOTHIAN

Publications Received

ZONE MELTING OF ORGANIC COMPOUNDS. By E. F. G. HERINGTON, D.Sc., A.R.C.S. Pp. viii + 162. Oxford: Blackwell Scientific Publications. 1963. Price 35s.

JOURNAL OF THE ASSOCIATION OF PUBLIC ANALYSTS. Volume 1, No. 1. First Quarter, 1963. Hon. Editor: Dr. E. C. WOOD. Pp. 24. London: The Association of Public Analysts. Annual Subscription for 4 quarterly parts 30s.; single copies 7s. 6d.

Orders, with remittance, should be sent direct to the Hon. Treasurer, Association of Public Analysts, Public Analyst's Laboratory, County Hall, Maidstone, Kent.

GAS CHROMATOGRAPHY ABSTRACTS 1962. Edited by C. E. H. KNAPMAN; Assistant Editor: D. AMBROSE. Pp. x + 195. · Sponsored by The Gas Chromatography Discussion Group of the Hydrocarbon Research Group of the Institute of Petroleum. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 42s.

BRITISH PHARMACOPOEIA 1963. Published under the Direction of the General Medical Council. Pp. xxviii + 1210. London: The Pharmaceutical Press. 1963. Price 100s.

DEVELOPMENTS IN APPLIED SPECTROSCOPY. Volume 2. Edited by J. R. FERRARO and J. S. ZIOMEK. Pp. x + 438. New York: Plenum Press Inc. 1963. Price $16.00.

BRITISH PHARMACEUTICAL CODEX, 1963. Published by direction of the Council of the Pharmceutical Society of Great Britain. Pp. xxxvi + 1433. London: The Pharamaceutical Press. 1963. Price 105s.

PRACTICAL ANALYTICAL METHODS FOR CONNECTIVE TISSUE PROTEINS. By J. E. EASTOE and A. COURTS. Pp. xiv + 145. London: E. & F. N. Spon Ltd. 1963. Price 42s.

SOLUBILITIES OF INORGANIC AND ORGANIC COMPOUNDS. Volume 1. BINARY SYSTEMS. Part I. Edited by Prof. H. STEPHEN, O.B.E., D.Sc., F.R.I.C., and Dr. T. STEPHEN, M.Sc., Ph.D. Pp. iv + 960. Oxford, London, New York and Paris: Pergamon Press. 1963. Price £10.

ANALYTICAL CHEMISTRY 1962. Edited by PHILIP W. WEST, A. M. G. MACDONALD and T. S. WEST. Pp. xii + 411. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 90s.

The Proceedings of the International Symposium held at Birmingham University (U.K.), April, 1962, in honour of Fritz Feig. Organised for the Society for Analytical Chemistry by its Midlands Section.

INTRODUCTION TO FLUORIMETRY. By A. H. GUNN. Pp. vi + 59. Surrey: Electronic Instruments Limited. 1963. Price 35s.

QUANTITATIVE ORGANIC ANALYSIS VIA FUNCTIONAL GROUPS. By SIDNEY SIGGIA, Ph.D. Third Edition. Pp. xiv + 697. New York and London: John Wiley & Sons Ltd. 1963. Price 145s.

ADVANCES IN MASS SPECTROMETRY. Volume 2. Edited by R. M. ELLIOTT. Pp. xviii + 628. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 140s.

Proceedings of a Conference held in Oxford, September 1961.

SPECTROMETRIC IDENTIFICATION OF ORGANIC COMPOUNDS. By ROBERT M. SILVERSTEIN and G. CLAYTON BASSLER. Pp. vi + 177. New York and London: John Wiley & Sons Ltd. 1963. Price 64s.

COMPREHENSIVE BIOCHEMISTRY. Volume 10. STEROLS, BILE ACIDS AND STEROIDS. Edited by MARCEL FLORKIN and ELMER H. STOTZ. Pp. xii + 212. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 55s.

VACUUM MICROBALANCE TECHNIQUES. Volume 3. Edited by KLAUS H. BEHRNDT. Pp. xvi + 215. New York: Plenum Press Inc. 1963. Price $9.50.

Proceedings of the Los Angeles Conference, October 30th, 1962.

RUSSIAN-ENGLISH CHEMICAL READER. By ALEX A. KRAMER. Pp. viii + 311. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Company Inc. 1963. Price 58s. 6d.

Reprints of "Methods of Separation of Long-chain Unsaturated Fatty Acids" by A. T. James

REPRINTS of the Review Paper "Methods of Separation of Long-chain Unsaturated Fatty Acids," by A. T. James published in this issue of *The Analyst* (pp. 572–582), will be available shortly from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not available through Trade Agents.

SEPTEMBER, 1963 Vol. 88, No. 1050

THE ANALYST

Report of the Analytical Methods Committee
1961/1962

THIS seventh Report of the Analytical Methods Committee of The Society for Analytical Chemistry reviews the progress of work during the two years 1961 and 1962, covering the period January 1st, 1961, to December 31st, 1962.

ANALYTICAL METHODS COMMITTEE

Chairman: D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C.
Boots Pure Drug Co. Ltd.

A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C.	*Analytical and Consulting Chemist (President of the Society)*
R. Belcher, Ph.D., D.Sc., F.Inst.F., F.R.I.C.*	*University of Birmingham (Professor of Analytical Chemistry)*
E. Bishop, B.Sc., A.R.C.S.T., F.R.I.C.	*University of Exeter (Department of Chemistry)*
L. Brealey, B.Sc., F.R.I.C.	*International Combustion Ltd.*
S. G. Burgess, B.Sc., Ph.D., F.Inst.Pet., F.Inst.S.P., F.R.I.C.	*Scientific Adviser, London County Council*
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Public Analyst, Official Agricultural Analyst and Consulting Chemist*
W. C. Johnson, M.B.E., F.R.I.C.	*Hopkin & Williams Ltd.*
A. G. Jones, B.Sc., F.R.I.C.†	*Imperial Chemical Industries Ltd. (Plastics Division)*
D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.	*Analytical and Consulting Chemist*
E. Q. Laws, B.Sc., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
D. T. Lewis, C.B., B.Sc., Ph.D., D.Sc., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist (Honorary Treasurer of the Society)*
R. F. Milton, B.Sc., Ph.D., M.I.Biol., F.R.I.C.	*Analytical and Consulting Biochemist*
R. M. Pearson, A.R.I.C.†	*Imperial Chemical Industries Ltd. (Billingham Division)*
A. A. Smales, O.B.E., B.Sc., F.R.I.C.	*Atomic Energy Research Establishment, Harwell*
R. E. Stuckey, Ph.D., D.Sc., F.P.S., F.R.I.C.	*Honorary Secretary of the Society*
C. Whalley, B.Sc., F.R.I.C.	*Laporte Chemicals Ltd.*
K. A. Williams, B.Sc., Ph.D., A.Inst.P., M.Inst.Pet., F.R.I.C.	*Analytical and Consulting Chemist*
H. N. Wilson, F.R.I.C.‡	*Imperial Chemical Industries Ltd. (Billingham Division)*

Secretary: Miss C. H. Tinker, B.Sc., Ph.D., A.R.I.C.

Assistant Secretary: Miss A. M. Parry, B.Sc. (Resigned November 1961)
Miss V. Lewis (Appointed November, 1962)

* Temporarily resigned May, 1961. † Appointed May, 1962. ‡ Resigned July, 1962.

GENERAL REVIEW

During 1961, the Committee was sorry to lose Dr. J. Haslam, who resigned on his retirement from Imperial Chemical Industries Ltd.; he had been a valuable member of the Committee for many years.

The Committee was also sorry to lose, through resignation to take up another appointment, the services of Miss A. M. Parry in November, 1961. Miss Parry had been Assistant Secretary to the Committee since January, 1956; she was succeeded, in November, 1962, by Miss V. Lewis.

PROGRESS OF WORK—

The Committee can once again report steady progress in its work. Some projects have been completed and the Sub-Committees and Panels, having prepared their Reports, which will be published during the coming year, have been disbanded. Two new committees were appointed during 1961. One of these is a Sub-Committee of the Analytical Methods Committee for investigating methods for Particle Size Analysis. This Sub-Committee had its first meeting in March, 1961, and, after nearly 2 years of vigorous work, it has prepared a comprehensive classification of methods that, it is hoped, will prove useful to chemists and physicists new to the subject and who require guidance on how to set about their task of finding suitable methods for their own particular types of materials and fields of application. This classification is being published in the March, 1963, issue of *The Analyst*. The other committee to be appointed is a new Panel of the Joint Committee of the Society (as represented by the Analytical Methods Committee) and The Pharmaceutical Society on Methods of Assay of Crude Drugs. This Panel, the seventh to be appointed by the Joint Committee, is investigating chemical methods for assaying the biologically active constituents of thyroid. An exploratory meeting was held on the subject in December, 1961, and the Panel had its first meeting in February, 1962. Unlike most sub-committees and panels of the A.M.C., which are commissioned to examine published or known methods of assay with a view to ensuring their applicability and reproducibility for reference purposes, this Panel has the more difficult task of devising a method practically *de novo*; for this purpose, its investigations are more in the nature of research on a collaborative basis.

The other existing Sub-Committees of the A.M.C. and Panels of the Joint Committee have, on the whole, been actively at work during the period under review. One of the Joint Committee's Panels—that dealing with the investigation of chemical methods for anthraquinone drugs (senna, cascara, etc.)—has resumed work again having been in suspension for nearly 2 years pending a parallel investigation on biological methods by an associated Panel (Panel 3A). Panel 3A has now unified a biological technique for senna fruit that can be used as a form of "yardstick" for correlating chemical methods with purgative activity in mice.

Another Panel of the Joint Committee—that on methods for lonchocarpus and derris (Panel 5)—completed its programme of work at the end of 1961 and its second Report, on the Colorimetric Determination of Rotenone, was published in *The Analyst* in November of that year. This Panel has now been disbanded.

The 1960 Report of the A.M.C. presented rather a gloomy picture with regard to the difficulties encountered by the Additives in Animal Feeding Stuffs Sub-Committee in its endeavour to apply known methods of analysis to animal and poultry feeds. It says much for all the members of the five Panels of that Sub-Committee that during the past two years these difficulties have been largely resolved, with the result that four of these Panels have completed their programmes and have been disbanded, and their Reports are to be published shortly. They include methods of assay for antibiotics (penicillin, chlortetracycline and oxytetracycline), for synthetic hormones (stilboestrol and hexoestrol) and for a number of vitamins (vitamin A and β-carotene, of the fat-soluble types, and vitamin B_{12}, nicotinic acid, pantothenic acid and riboflavin, of the water-soluble group of B-vitamins). Only the methods for two of the B-vitamins—choline and pyridoxin—have proved to be somewhat intractable, and it became evident that these would require lengthy and specialised investigation; some of the problems encountered are described later (see p. 665).

The fifth Panel of this Sub-Committee—that on prophylactics—has completed its work on one item, nitrofurazone, and has prepared its Report, which will be published with those on the other additives. In view of this Panel's lengthy programme, it has been decided that it should be re-organised as a Sub-Committee in its own right directly under the aegis of the A.M.C. This arrangement has enabled the Additives Sub-Committee to wind up its commitments, and it has been disbanded.

Associated with the Additives Sub-Committee is another Sub-Committee that dealt, independently, with methods for determining trace elements in fertilisers and feeding stuffs. This latter committee started its work in 1957—about a year and a half before that on additives—and it has now completed its lengthy programme of work; the collected methods for about fourteen elements are to be published early in 1963 by the Society as a separate booklet, since it is envisaged that they will be capable of application to products other than fertilisers and feeding stuffs.

Another Sub-Committee to complete part of its programme is that on Metallic Impurities in Organic Matter. In 1960, it published two Reports, on Small Amounts of Arsenic and on Methods for the Destruction of Organic Matter; during 1961, the work on a method for copper was completed and the Report is being published in the April, 1963, issue of *The Analyst*. Meanwhile, the Sub-Committee has continued with its programme and has been considering methods for zinc and mercury; after examining its specificity in the presence of certain other metals that react with dithizone, the Sub-Committee can recommend the method for zinc adopted by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee. The determination of very small amounts of mercury in organic matter has presented a problem to analysts for a long time, since the removal of the organic matter can frequently lead to a significant loss of the metal. It is possible that radiochemical procedures (particularly isotope dilution), which are rapidly increasing in popularity and becoming more available to smaller laboratories, might be used as the basis of a reference method for mercury, and the Sub-Committee has been considering this possibility. However, because some laboratories may not have the necessary facilities for some time, the Sub-Committee thinks that a chemical method should be investigated in the first place, and collaborative work is proceeding on a method that appears to be promising and that is sensitive for measuring amounts down to half a microgram. During this collaborative work, use is being made of radiochemical techniques for monitoring the various stages during the wet-combustion procedure to check that there is no loss of mercury.

Two Reports were published in the September, 1961, issue of *The Analyst* by the Meat Products Sub-Committee. The first Report, on Nitrogen Factors for Pork, presents a vast amount of evidence from results collected from laboratories in the United Kingdom and in Europe to show that the hitherto-accepted factor of 3·6 for the conversion of meat nitrogen into meat content was too high for pork. This had meant that in the past manufacturers of pork products often had to put in more meat than necessary to ensure that analysis of their products would give results within the statutory limits; the factor of 3·45, as recommended in the Report, appears to be the best compromise on the evidence of the results presented, and it has been adopted for use by the Association of Public Analysts and by the meat trade. The second Report is a short one dealing with the Nitrogen Content of Rusk Filler. Since rusk filler is used in the manufacture of sausages and some other meat products, it was considered necessary to revise the value for its nitrogen content in view of changes that have occurred in milling practice since the second World War. The Sub-Committee is continuing its programme of work by collecting values for the nitrogen contents of beef and of chicken meat, in a similar manner to that carried out for pork. Its Report on the Nitrogen Factors for Beef has been completed and will be published in 1963, and its Report on chicken meat is being prepared.

The Chlorine in Organic Compounds Sub-Committee completed its programme of work during 1962, and its Report on the application of the oxygen-flask combustion method to the determination of pesticides containing organically-bound chlorine will be published in 1963.

It was reported in 1960 that two more Panels (set up jointly by The Society for Analytical Chemistry, the Association of British Manufacturers of Agricultural Chemicals, and the Scientific Sub-Committee of the Interdepartmental Advisory Committee on Poisonous Substances used in Agriculture and Food Storage) had been set up to study methods for determining malathion and organo-mercury residues. The Report on malathion was published in *The Analyst* at the end of 1960, and that on organo-mercury appeared in September, 1961. Another Panel set up in 1960 was that on demeton-methyl residues in fruits and vegetables; its Report was published in *The Analyst* in June, 1962.

The Joint Committee of The Pharmaceutical Society and The Society for Analytical Chemistry (represented by the Analytical Methods Committee) has continued its work during the past 2 years and, as mentioned earlier, the Panel on Lonchocarpus and Derris has been disbanded on completion and publication of its second Report. Also, as mentioned earlier, the new Panel on Thyroid (Panel 7) has now been at work for nearly a year. The Panel engaged on methods for the chemical assay of the Capsaicin Content of Capsicum and its Preparation has completed its programme of work and is preparing its second Report, the first being published in 1959. The first Report included recommended methods for the assay of the drug and for three official preparations (oleoresin, tincture and ointment). Since then, experience of the methods has revealed the need for some revisions and these are included in the second Report, together with a simpler method for ointment; a more practicable colorimetric method, involving use of Gibb's phenol reagent, has been devised for end-determination (the spectrophotometric difference method remaining the method of choice) and is also included.

Other Sub-Committees of the A.M.C. and Panels of the Joint Committee have continued with their programmes of work. Details of individual committees—their personnel and reports of work—will be found below.

S.A.C. SCHOLARSHIP—

Dr. J. H. Stevenson was appointed in October, 1960, to carry out investigation into bio-assay methods for determining pesticide residues, at Rothamsted Experimental Station. He was unable to continue his work for a second year, and his results are now awaiting publication.

ANALYTICAL METHODS TRUST—

The second of the three-year periods for the receipt of promised subscriptions to the Trust Fund ended in 1960 and, as in 1958, an Appeal to Industry to continue its financial aid of the work of the Analytical Methods Committee was made in June, 1961. The response to this appeal was extremely gratifying; 76 organisations promised donations, some of which were by Deed of Covenant for 7 years, others for 3 years and some others for 1961 only. This represented an increase of 51 over the number in 1960 and showed the wider response to the appeal.

The total amount received for 1961 was £5,665, and firm promises totalling £3,646 were made for each of the years 1962 and 1963.

ANNUAL ACCOUNTS—

The audited statements of accounts for the two financial years ending October 31st, 1961, and October 31st, 1962, are shown in Appendixes I and II, respectively.

REPORTS OF SUB-COMMITTEES OF THE ANALYTICAL METHODS COMMITTEE

PUBLICATIONS SUB-COMMITTEE

CONSTITUTION—

D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C. (*Chairman*)	*Chairman, Analytical Methods Committee*
J. B. Attrill, M.A., F.R.I.C.	*Editor*, The Analyst
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Member, Analytical Methods Committee*
D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.	*Member, Analytical Methods Committee*
H. N. Wilson, F.R.I.C.*	*Member, Analytical Methods Committee*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C. (*Secretary and Editor*)	*Editor, Scientific Publications, The Pharmaceutical Society of Great Britain*

* Resigned July, 1962.

PROGRESS OF WORK—

Much progress has been made in the collection and editing of all those recommended methods of analysis published since 1926 by the Analytical Methods Committee in its various Reports. These collected methods were published in March, 1963, under the title "Official, Standardised and Recommended Methods of Analysis," and included a revised and expanded version of the Bibliography of Standard and Recommended Methods originally published in 1951.

ADDITIVES IN ANIMAL FEEDING-STUFFS SUB-COMMITTEE

CONSTITUTION—

D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C.	*Analytical and Consulting Chemist*
W. T. Barker*	*Ministry of Agriculture, Fisheries and Food*
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Public Analyst, Official Agricultural Analyst and Consulting Chemist*
R. F. Phipers, B.Sc., Ph.D.	*Cooper Technical Bureau*
S. A. Price, B.Sc., F.R.I.C.	*Vitamins Ltd.*
C. J. Regan, B.Sc., F.R.I.C.	Formerly *Chemist-in-Chief, London County Council*
W. L. Sheppard, F.R.I.C.	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
R. E. Stuckey, Ph.D., D.Sc., F.P.S., F.R.I.C.	*British Drug Houses Ltd.*
F. R. Williams†	*Ministry of Agriculture, Fisheries and Food*
Miss C. H. Tinker (*Secretary*)	

* Appointed May, 1962. † Resigned May, 1962.

TERMS OF REFERENCE—"To investigate and prepare methods for determining the amounts of additives (nutrients, stimulants and prophylactics) in animal and poultry feeding stuffs."

PROGRESS OF WORK—

Although the task was expected to prove difficult when originally undertaken by the Sub-Committee, the efforts of its Panels have led to a resolution of most of the problems. The Antibiotics, Hormones and the Vitamins (fat-soluble) Panels have completed their programmes; the Vitamins (water-soluble) Panel has completed its work on all but two of the vitamins listed in its programme. The various Reports by both these groups, giving recommended methods of assay, are being submitted for publication. The Prophylactics Panel has completed its Report on Nitrofurazone.

Since the remaining projects require a considerable amount of further investigation, either collaboratively or by individual research, it was decided to disband the Sub-Committee, which had acted purely in a steering and advistory capacity for all the Panels. This has now been done, and the opportunity is taken here to thank the members, not only of the Sub-Committee itself, but of all the Panels, for the vast amount of hard work that has been so willingly undertaken. Only the Prophylactics Panel survives to carry on its programme of work, and, to allow of its re-organisation and broadening of scope, it has been decided to reconstitute it as a Sub-Committee of the Analytical Methods Committee in its own right.

During the work of the Additives Sub-Committee it has become apparent that, in some instances, wide tolerances on the assay must be allowed; an unrealistic amount of extra work would be necessary to increase the accuracy. Throughout the work, the problem of sampling has been apparent and the Sub-Committee emphasises that the stated accuracy of a recommended method can be attained only if the analyst's sample is properly prepared; problems involved in sampling the bulk material are, however, not within the Sub-Committee's province.

ANTIBIOTICS PANEL

CONSTITUTION—

S. A. Price, B.Sc., F.R.I.C. (*Chairman*)	*Vitamins Ltd.*
A. J. Cavell, M.Sc., A.R.C.S., D.I.C., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
Mrs. J. Gammon, B.Sc.	Formerly *Cyanamid of Great Britain Ltd., Lederle Laboratories*
O. Hughes	*Pfizer Ltd.*
W. P. Jones, F.P.S., F.R.I.C.	*Cyanamid of Great Britain Ltd., Lederle Laboratories*
G. Sykes, M.Sc., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
J. H. Taylor, Ph.D., M.R.C.V.S.	*Cyanamid of Great Britain Ltd., Agricultural Division*
S. Varsanyi, A.I.S.T.	*Glaxo Laboratories Ltd.*
Miss A. M. Parry (*Secretary*)	

PROGRESS OF WORK—

The Panel has completed its programme of work and its Report will be published in 1963. Methods for the three permitted antibiotics (penicillin, chlortetracycline and oxytetracycline) can now be recommended for the assay of feeding stuffs, with the limitation that the identity of the antiobiotic is known and also that only *one* antibiotic is present.

Both supplements and supplemented feeding stuffs are covered. Because of the higher levels of antibiotics in the former (a few grams per pound), chemical methods of assay are recommended; for the much lower levels in feeds (a few grams per ton), microbiological methods are required.

HORMONES PANEL

CONSTITUTION—

R. E. Stuckey, Ph.D., D.Sc., F.P.S., F.R.I.C. (*Chairman*)	*British Drug Houses Ltd.*
J. Allen, A.R.I.C.	*British Drug Houses Ltd.*
L. Brealey, B.Sc., F.R.I.C.	Formerly *Boots Pure Drug Co. Ltd.*
J. A. Potter, A.R.I.C.	*Analytical and Consulting Chemist*
W. L. Sheppard, F.R.I.C.	Formerly *Unilever Ltd.*
Miss A. M. Parry (*Secretary*)	

Progress of work—

The Panel has completed its programme of work and its Report will be published in 1963. Only the two synthetic hormones, stilboestrol and hexoestrol, have been investigated and physico-chemical methods are recommended.

PROPHYLACTICS PANEL

Constitution—

R. F. Phipers, B.Sc., Ph.D. (*Chairman*)	*Cooper Technical Bureau*
C. W. Ballard, B.Sc., F.P.S., F.R.I.C.	*May & Baker Ltd.*
N. C. Brown, M.A., B.Sc., A.R.I.C.	*Cooper Technical Bureau*
H. G. Dickenson, B.Sc., Ph.D.*	*Ward, Blenkinsop & Co. Ltd.*
G. Drewery, B.Sc., F.R.I.C.†	*Merck Sharp & Dohme Ltd.*
A. W. Hartley, F.R.I.C.	*Spillers Ltd.*
A. Holbrook, F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Pharmaceuticals Division*)
D. H. Mitchell‡	*Wellcome Chemical Works*
S. G. E. Stevens, B.Sc., F.R.I.C.	*Smith Kline & French Laboratories Ltd.*
J. A. Stubbles, B.Sc.	*May & Baker Ltd.*
Miss A. M. Parry Miss C. H. Tinker (*Secretaries*)	

* Corresponding member. † Appointed January, 1962. ‡ Appointed April, 1961.

Progress of work—

The Panel has a long programme of work, complicated by the fact that the fashion in prophylactic chemicals is constantly changing, so that new substances are frequently appearing and others are likely to go out of fashion before there has been time to devise a suitable method for determining them in feeds.

A method for nitrofurazone can now be recommended, and the Panel's Report on this will be published in 1963. The work on methods for acinitrazole, sulphaquinoxaline and Amprolium is virtually completed, and Reports are to be prepared for publication.

As mentioned above, the future work of the Panel is to be continued by the "Prophylactics in Animal Feeds Sub-Committee," which is being formed from the Panel members with additional representation from other organisations interested in the subject; Dr. Phipers will continue as Chairman of the new Sub-Committee.

VITAMINS (FAT-SOLUBLE) PANEL

Constitution—

W. L. Sheppard, F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
C. R. Louden, B.Sc., F.R.I.C.	*R. Silcock & Sons Ltd.*
H. Pritchard, M.Sc., F.R.I.C.	*Analytical and Consulting Chemist*
S. A. Reed, B.Sc., A.R.I.C.	*British Cod Liver Oils* (*Hull and Grimsby*) *Ltd.*
G. Whalley, B.Sc., F.R.I.C.	*Unilever Ltd.*
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

Progress of work—

As reported 2 years ago, the Panel has completed its work on the spectrophotometric method for both vitamin A and for β-carotene, and the Report has been approved for publication. In addition, a colorimetric method has since been tested collaboratively and is to be included in the Report as a quick routine method; however, it is not sufficiently reliable to recommend it as a reference method. The Panel has now been disbanded.

VITAMINS (WATER-SOLUBLE) PANEL

CONSTITUTION—

A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Analytical and Consulting Chemist*
J. E. Ford, B.Sc., Ph.D.	*National Institute for Research in Dairying*
B. M. Gibbs, B.Sc., A.R.I.C.	*Unilever Ltd.*
F. W. Norris, Ph.D., D.Sc., A.R.C.S., D.I.C., F.R.I.C.	*University of Birmingham (Department of Biochemistry)*
S. A. Price, B.Sc., F.R.I.C.	*Vitamins Ltd.*
H. Pritchard, M.Sc., F.R.I.C.	*Analytical and Consulting Chemist*
F. Clermont Scott, B.Sc., F.R.I.C.	*Vitamins Ltd.*
S. Varsanyi, A.I.S.T.	*Glaxo Laboratories Ltd.*
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

PROGRESS OF WORK—

The Panel has studied and tested collaboratively on animal feeding stuffs selected methods of assaying riboflavin, nicotinic acid, vitamin B_{12}, pantothenic acid, pyridoxin and choline. Because of their specificity and sensitivity, microbiological procedures have been the methods of first choice.

It has been possible to determine microbiologically nicotinic acid, vitamin B_{12}, pantothenic acid and riboflavin in unsupplemented and supplemented animal feeding stuffs with an acceptable "between-laboratories" variance, and the Panel's two reports on recommended methods for these four vitamins has been approved for publication. During the Panel's earlier investigation of the determination of riboflavin by the microbiological method recommended by the Analytical Methods Committee for foods (*Analyst*, 1946, **71**, 397), it was suggested that the unacceptable divergence between the results obtained in different laboratories was attributable to the extraction procedure. As a result of a study of the effect of variations in the method of extraction, it has been possible to introduce modifications that allow the riboflavin activity of animal feeding stuffs to be determined with an accuracy commensurate with those of the assay methods recommended for the other three vitamins. Collaborative studies of a microbiological method involving *S. zymogenes* have revealed that, despite its greater sensitivity, the method has no advantage over the recommended method, which relies on *L. helveticus* as the test organism.

Continued studies of the remaining two vitamins on the Panel's original list—namely, pyridoxin and choline—have failed to produce methods of assay that can be recommended for use with animal feeding stuffs. Several microbiological methods of assaying pyridoxin have been subjected to collaborative trials, but none has given results with an acceptable "between-laboratories" variance. Collaborative studies of microbiological and chemical methods of assaying choline have been no more successful.

ANALYTICAL STANDARDS SUB-COMMITTEE

CONSTITUTION—

E. Bishop, B.Sc., A.R.C.S.T., F.R.I.C. (*Chairman*)	*University of Exeter (Department of Chemistry)*
S. Andrus, A.R.I.C.	*Laporte Chemicals Ltd.*
P. R. W. Baker, M.Sc., A.R.I.C.	*Wellcome Research Laboratories*
A. G. Hill, F.R.I.C.	*British Drug Houses Ltd.*
R. M. Pearson, F.R.I.C.	*Imperial Chemical Industries Ltd. (Billingham Division)*
J. M. Skinner, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd. (Billingham Division)*
W. I. Stephen, B.Sc., Ph.D., A.R.I.C.	*University of Birmingham (Department of Chemistry)*
N. E. Topp, B.Sc., Ph.D., A.R.I.C.*	*National Chemical Laboratory*
J. T. Yardley, B.Sc., F.R.I.C.	*Hopkin & Williams Ltd.*
Miss A. M. Parry Miss C. H. Tinker (*Secretaries*)	

* Appointed April, 1961.

TERMS OF REFERENCE—"To examine existing analytical standards and to select suitable substances."

PROGRESS OF WORK—

Continuing the critical assessment of standards for acid - base titrimetry with a view to recommending a suitable standard to IUPAC, the Sub-Committee has set up a carefully defined scale of standards. After a detailed appraisal of the material submitted for consideration, sodium carbonate was selected for initial experimental examination in view of new work on the preparation of this compound. A statistical design was set up for a collaborative assay involving four laboratories, four samples of sodium carbonate prepared by two different methods and two reference standards—atomic-weight silver and zone-refined benzoic acid. Experimental procedures were devised to give the required precision of ±0·1 per cent., and have been tested. The first collaborative assay broke down over the difficulty of storing and transporting molar hydrochloric acid without change of concentration; this effect was later discovered to be due mainly to leakage through the stoppers of polythene bottles. The second attempt failed because of the difficulty of removing carbon dioxide from solution without simultaneous loss of traces of benzoic acid. The third assay, in which the sodium carbonate samples were related to a uniform sample of atomic-weight silver through individual hydrochloric acid samples, met with considerable success, but revealed an interesting laboratory bias of ±0·02 per cent. Although the result of this assay fell just within the prescribed limits, a fourth and final assay on fresh samples was made, and confirmed that the samples were assaying at about 99·96 per cent. The varieties of sodium carbonate comprising the test samples did not therefore meet the requirements for a primary standard. Nevertheless, confirmation was received that the methods of assay developed for this work were giving adequate replication and that certain modifications and alternatives also reached this standard. Other sources of sodium carbonate were examined, and a fifth collaborative assay showed that samples assaying at 99·996 per cent. were available. Meanwhile, attempts were made to trace the source of the 0·04 per cent. deficiency in the earlier samples, but were met with only partial success. The latest assays, showing a scatter of 18 parts per million over 35 analyses, were regarded as satisfactory evidence that the proposed methods were suitable and that sodium carbonate was an appropriate primary standard for the calibration of solutions of strong acids. Finally, a readily accessible source of sodium carbonate, on which preliminary assays are promising, is being investigated. A final report, embodying the recommendation of sodium carbonate, together with the method of assay, is being prepared and will be made available to the IUPAC Conference in July, 1963.

A detailed examination of losses of benzoic acid has also been made, with the conclusion that such losses come within the experimental error and can readily be determined. The scatter of the assay results, however, of 200 parts per million indicate the unsuitability of benzoic acid for applications to titrimetry, although the Sub-Committee entertains no doubts as to its purity and suitability in other applications.

The possibilities of using the coulomb as a universal reference standard are being explored, and a proposal has been made to the Analytical Methods Committee that specialised research work on this project would be well worthwhile.

CHLORINE IN ORGANIC COMPOUNDS SUB-COMMITTEE

CONSTITUTION—

R. Belcher, Ph.D., D.Sc., F.Inst.F., F.R.I.C. (*Chairman*)	*University of Birmingham (Professor of Analytical Chemistry)*
J. H. Dunn, B.Sc., A.R.I.C.	*Plant Protection Ltd.*
K. Gardner, B.Sc., F.R.I.C.	*Fisons Pest Control Ltd.*
R. Goulden, F.R.I.C.	*"Shell" Research Ltd.*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
Miss A. M. G. Macdonald, M.Sc., Ph.D., A.R.I.C.	*University of Birmingham (Department of Chemistry)*
Miss C. H. Tinker (*Secretary*)	

TERMS OF REFERENCE—"To prepare methods for the determination of organically-bound chlorine, having special reference to commercial preparations such as pesticides."

PROGRESS OF WORK—

The work of the Sub-Committee has now been completed, and its Report will be published in 1963. The method recommended is based on the oxygen-flask combustion technique, and it has been tested collaboratively on (*a*) chlorobenzoic acid, (*b*) dieldrin 50 per cent. water-dispersible powder and (*c*) a miscible oil containing pentachlorophenol, by both the members of the Sub-Committee and other analysts who had not had experience of the technique. Results were excellent and the method can be accepted with confidence as a standard method for all but low-concentrate pesticide dusts, in which the high proportion of inorganic filler prevents complete combustion; for this latter type of product the Stepanow method should be used.

ESSENTIAL OILS SUB-COMMITTEE

CONSTITUTION—

G. W. Ferguson, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Analytical and Consulting Chemist*
A. J. M. Bailey, B.Sc., F.P.S., F.R.I.C.	*W. J. Bush & Co. Ltd.*
H. E. Brookes, B.Sc., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
K. Field, M.Sc., Ph.D.*	*D.S.I.R., Laboratory of the Government Chemist*
D. Holness, B.A.	*Proprietary Perfumes Ltd.*
H. T. Islip, B.Sc., F.R.I.C.	Formerly *Tropical Products Institute*
P. McGregor, B.Sc., A.H.-W.C., F.R.I.C.†	*D.S.I.R., Laboratory of the Government Chemist*
T. L. Parkinson, B.Sc., Ph.D., F.R.I.C.	*Beecham Foods Ltd.*
Miss H. M. Perry, M.Sc., F.R.I.C.	*Stafford Allen & Sons Ltd.*
G. B. Pickering, M.A., B.Sc., Ph.D., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
J. H. Seager, M.Sc., F.R.I.C.	*Yardley & Co. Ltd.*
S. G. E. Stevens, B.Sc., F.R.I.C.	*Smith Kline & French Laboratories Ltd.*
B. D. Sully, B.Sc., Ph.D., A.R.C.S., F.R.I.C.	*A. Boake, Roberts & Co. Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed July, 1962. † Resigned July, 1962.

PROGRESS OF WORK—

Studies of methods for the evaluation of specific groups of substances in essential oils have been continued. Completion of Mr. Holness's study of the reactions of citronellol and geraniol, on formylation, and the publication of his Report (*Analyst*, 1961, **86**, 231) provided a major contribution to the proceedings of the I.S.O. meeting on Essential Oils at The Hague in May, 1961. The van Os and Elema method for determining geraniol and citronellol when these occur together has also been studied, as have been alternative methods of acylation of alcohols. However, the Sub-Committee is now much in favour of Dr. Sully's stearoylation method (*Analyst*, 1962, **87**, 940) for the determination of hydroxyl groups, and a series of collaborative tests is at present being carried out.

Investigations into the determination of carbonyl groups have continued, and a preliminary study has been made of spectrophotometric methods that have been published for the determination of citral as α,β-unsaturated aldehyde. Work has continued on the determination of phenols and some collaborative tests have been made on Demetrius and Sinsheimer's method; it is hoped that a short Report on these investigations will be published during the coming year.

MEAT PRODUCTS SUB-COMMITTEE

CONSTITUTION—

S. M. Herschdoerfer, Ph.D., F.R.I.C. (*Chairman*)	*T. Wall & Sons (Ice Cream) Ltd.*
S. Back, B.Sc., F.R.I.C.	*Crosse & Blackwell Ltd.*
P. O. Dennis, B.Sc., F.R.I.C.	*Oxo Ltd.*
J. R. Fraser, B.Sc., A.C.G.F.C., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
H. C. Hornsey, F.R.I.C.	*J. Sainsbury Ltd.*
A. J. Kidney, B.Sc., Ph.D., A.R.C.S., A.R.I.C.*	*T. Wall & Sons (Meat and Handy Foods) Ltd.*
R. A. Lawrie, B.Sc., Ph.D., F.R.I.C.	*A.R.C., Low Temperature Research Station*
T. McLachlan, D.C.M., A.C.G.F.C., M.I.Biol., F.R.I.C.†	*Public Analyst*
A. McM. Taylor, B.Sc., Ph.D., F.R.I.C.	*British Food Manufacturing Industries Research Association*
E. F. Williams, M.A., F.R.I.C.	*J. Sainsbury Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed August, 1961. † Appointed June, 1962.

TERMS OF REFERENCE—"(*a*) the determination of the meat content of products containing meat; (*b*) the determination of the constituents of meat and meat products.

NOTE—The term 'meat products' to include hydrolysed protein and, if found necessary, fish pastes."

PROGRESS OF WORK—

The Sub-Committee's Reports on Nitrogen Factors for Pork and on Nitrogen Content of Rusk Filler were published in *The Analyst* in September, 1961. Work has continued on the collection, on similar lines, of values for the nitrogen contents of beef and chicken meat. The Sub-Committee's Reports on beef and chicken factors will be published in 1963.

METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

CONSTITUTION—

W. C. Johnson, M.B.E., F.R.I.C. (*Chairman*)	*Hopkin & Williams Ltd.*
J. C. Gage, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Industrial Hygiene Laboratories*)
T. T. Gorsuch, B.Sc., Ph.D., A.R.I.C.	*U.K. Atomic Energy Authority, The Radiochemical Centre*
Miss E. M. Johnson, M.Sc., A.R.I.C.	*British Food Manufacturing Industries Research Association*
R. F. Milton, B.Sc., Ph.D., M.I.Biol., F.R.I.C.	*Analytical and Consulting Biochemist*
E. J. Newman, B.Sc., F.R.I.C.	*Hopkin & Williams Ltd.*
W. G. Sharples, A.R.I.C.	*Imperial Chemical Industries Ltd.* (*Dyestuffs Division*)
G. B. Thackray, B.Sc., F.R.I.C.*	*Staffordshire County Council*
E. I. Johnson, M.Sc., F.R.I.C.†	*D.S.I.R., Laboratory of the Government Chemist*
J. F. C. Tyler, B.Sc., Ph.D., A.R.I.C.‡	*D.S.I.R., Laboratory of the Government Chemist*
Miss A. M. Parry Miss C. H. Tinker } (*Secretaries*)	

* Appointed May, 1961. † Appointed May, 1962. ‡ Resigned May, 1962.

TERMS OF REFERENCE—"To investigate the determination of small quantities of metals in organic matter."

PROGRESS OF WORK—

The Sub-Committee completed its work on the determination of trace amounts of copper in organic matter, and its Report will be published in 1963. The method for zinc adopted by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee has been accepted as being applicable to organic matter generally, and the specificity of the method for zinc in the presence of certain other metals that react with dithizone has been confirmed.

The rather special problems attending the determination of mercury in organic matter have been considered at some length, and a programme of individual and collaborative work is in hand; use is being made of radiochemical techniques to check any losses of mercury during the various stages of the procedure.

DIRECT MICRO-DETERMINATION OF OXYGEN IN ORGANIC MATTER SUB-COMMITTEE

CONSTITUTION—

D. W. Wilson, M.Sc., F.R.I.C. (*Chairman*)	*Sir John Cass College* (*Department of Chemistry*)
P. R. W. Baker, M.Sc., A.R.I.C.	*Wellcome Research Laboratories*
Miss B. B. Bauminger, Ph.D., A.I.R.I., F.R.I.C.	*Dunlop Research Centre*
W. T. Chambers, B.Sc., Ph.D., A.R.I.C.	*British Rubber Producers' Research Association*
A. F. Colson, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Alkali Division*)
Miss M. Corner, B.Sc., F.R.I.C.*	*D.S.I.R., National Chemical Laboratory*
Miss J. Cuckney	*Imperial College of Science and Technology* (*Department of Chemistry*)
F. Ellington, B.Sc., A.R.C.S., F.R.I.C.	*National Coal Board, Coal Research Establishment*
F. J. McMurray	*Wellcome Chemical Works*
M. P. Mendoza, B.Sc., A.R.C.S.	*British Coal Utilization Research Association*
F. H. Oliver	*Parke, Davis & Co.*
H. J. Warlow	*D.S.I.R., Tropical Products Institute*
C Whalley, B.Sc., F.R.I.C.	*Laporte Chemicals Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Deceased November, 1962.

TERMS OF REFERENCE—"To investigate the Unterzaucher method, and its modifications, for the micro-determination of oxygen."

PROGRESS OF WORK—

No progress can be reported since this Sub-Committee has been in enforced abeyance since 1960.

PARTICLE SIZE ANALYSIS SUB-COMMITTEE

CONSTITUTION—

E. Q. Laws, B.Sc., F.R.I.C. (*Chairman*)	*D.S.I.R., Laboratory of the Government Chemist*
R. de B. Ashworth, M.Sc., Ph.D., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory*
D. G. Beech, Ph.D.	*British Ceramic Research Association*
C. G. L. Furmidge, B.Sc., Ph.D., A.R.I.C.	*"Shell" Research Ltd.*
H. Heywood, Ph.D.	*Director, Woolwich Polytechnic*
H. W. Hibbott	*D. R. Collins Ltd.*
J. F. Hinsley, F.I.M.*	*Edgar Allen & Co. Ltd.*
R. Howes, B.Sc.	*Chesterford Park Research Station*
R. Jackson, Ph.D.	*British Coal Utilisation Research Association*
B. H. Kaye, B.Sc., Ph.D.	*Research Council of the British Whiting Federation* (Formerly with *Nottingham & District Technical College*)
Miss C. H. Tinker (*Secretary*)	

* Appointed March, 1962.

TERMS OF REFERENCE—"To study methods of particle size analysis, to survey available instruments and to evaluate them with regard to their principles of operation and fields of application."

PROGRESS OF WORK—

The Sub-Committee held its first meeting in March, 1961, and its initial task has been to list and classify existing methods for determining particle size in the sub-sieve range (*i.e.*, below 76 μ). After 2 years' hard work this classification, comprising 74 methods, has been completed and is being published as a Review article in *The Analyst* in March, 1963. The principle of each method is briefly described and accompanied by adequate literature references.

The second, and more difficult, stage of the Sub-Committee's work will consist of some form of appraisement or evaluation of methods or apparatus, and its is envisaged that, since this will entail collaborative tests of a wide variety of apparatus, the constitution of the Sub-Committee will be supplemented by co-opted members, as and when necessary for special tests.

TRACE ELEMENTS IN FERTILISERS AND FEEDING STUFFS SUB-COMMITTEE

CONSTITUTION—

C. J. Regan, B.Sc., A.R.I.C. (*Chairman*)	Formerly *Chemist-in-Chief, London County Council*
S. M. Boden, B.Sc., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
S. G. Burgess, B.Sc., Ph.D., F.Inst.Pet., M.Inst.S.P., F.R.I.C.	*Scientific Adviser, London County Council*
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Public Analyst, Official Agricultural Analyst and Consulting Chemist*
E. I. Johnson, M.Sc., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
R. F. Milton, B.Sc., Ph.D., M.I.Biol., F.R.I.C.	*Analytical and Consulting Biochemist*
R. L. Mitchell, B.Sc., Ph.D., F.R.I.C., F.R.S.E.	*Macaulay Institute for Soil Research*
J. B. E. Patterson, M.Sc., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
W. L. Sheppard, F.R.I.C.	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

TERMS OF REFERENCE—"To devise appropriate methods of analysis (to be recommended for inclusion in the Regulations under the Fertilisers and Feeding Stuffs Act, 1926) for the determination of boron, cobalt, copper, fluorine, iodine, iron, magnesium, molybdenum, selenium and zinc, which can be expected to be present in fertilisers and feeding stuffs."

PROGRESS OF WORK—

By the end of 1961, the first draft of the final Report had been prepared; this comprised an introductory section, a section on general considerations and then detailed procedures for the preparation of the sample and for the determination of the eleven elements listed above. In addition, methods for chromium and nickel were added to cater for fertilisers containing sewage sludge; methods for calcium and chloride ion were also included as a result of a request from the Additives in Animal Feeding Stuffs Sub-Committee, of which this Sub-Committee acted as the Minerals Panel. For several of the elements, alternative methods are given for application in various circumstances. Publication of these collected methods is to be in the form of a separate booklet, under the title "Determination of Trace Elements, with Special Reference to Fertilisers and Feeding Stuffs," since it is envisaged that the methods will find a wider application than that for which they were originally intended; the book is being published early in 1963.

REPORT OF THE P.S. - S.A.C. JOINT COMMITTEE ON METHODS OF ASSAY OF CRUDE DRUGS

MAIN COMMITTEE

CONSTITUTION—

K. R. Capper, Ph.D., B.Pharm., F.P.S., D.I.C. (*Chairman*)	*Pharmaceutical Society of Great Britain*
J. Allen, A.R.I.C.*	*British Drug Houses Ltd.*
T. C. Denston, B.Pharm.†	*British Pharmacopoeia Commission*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.	*University of London (Professor of Pharmacognosy)*
A. J. Feuell, B.Sc., Ph.D., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C.	*Chairman, Analytical Methods Committee of the S.A.C.*
R. Higson, F.P.S.	*Ministry of Health (Supplies Branch)*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
H. C. Macfarlane, A.R.T.C.S., F.R.I.C.‡	*Analytical and Consulting Chemist*
W. Mitchell, B.Sc., Ph.D., F.R.I.C.	*Stafford Allen & Sons Ltd.*
R. F. Phipers, B.Sc., Ph.D.	*Cooper Technical Bureau*
J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S.	*Bradford Institute of Technology (Department of Pharmacy)*
D. Watt, F.P.S.	*T. & H. Smith Ltd.*
Miss C. H. Tinker (*Secretary*)	
Miss A. M. Parry (*Assistant Secretary*)§	

* Appointed February, 1962. † Appointed February, 1962.
‡ Resigned October, 1962. § Resigned November, 1961.

TERMS OF REFERENCE—"To prepare standard methods of assay of crude drugs and kindred materials."

PROGRESS OF WORK—

The Main Committee, in its steering capacity, is able to report continued progress in most of its Panels. Since its establishment in 1956, eight Panels have been set up to examine methods of assay of different drugs that are widely used but have no official or recognised assay procedure. During the past 2 years, one panel has been disbanded on completion of its programme of work and one new Panel has been set up; two other Panels have been in abeyance pending the outcome of associated work elsewhere.

Panel 5 (Lonchocarpus and Derris) was disbanded during 1961 on completion of its second Report—The Colorimetric Determination of Rotenone—which was published in *The Analyst* in November, 1961. Mention has been made earlier of the work of the new Panel on chemical methods for Thyroid (Panel 7), and further details of its progress and problems are given below.

Panel 1 (Digitalis Purpurea) was suspended in 1959 because no further progress could be made on a collaborative basis until considerable individual research had been undertaken. Since the problems encountered by the Panel have not yet been resolved elsewhere, it has has been decided to disband it; it is hoped, however, that it will be reconstituted at some later date. The other Panel that has been in suspension since 1960 is that dealing with chemical methods for anthraquinone drugs (Panel 3); as mentioned earlier this Panel began work again in December, 1962.

The three remaining Panels have continued with their programmes of work, and one of them—Panel 2 (Capsicum—Capsaicin Content)—is preparing its second, and final, Report.

PANEL 2: CAPSICUM—CAPSAICIN CONTENT

CONSTITUTION—

H. B. Heath, M.B.E., B.Pharm. (*Chairman*)	*Stafford Allen & Sons Ltd.*
C. F. G. Fost, M.P.S.	*W. J. Bush & Co. Ltd.*
C. A. Macdonald, B.Sc., F.R.I.C.	*Evans Medical Research Laboratories*
E. A. Elsbury, F.R.I.C.†	*Parke, Davis & Co.*
A. J. Middleton, B.Pharm., M.P.S., A.R.I.C.*	*Parke, Davis & Co.*
G. R. A. Short, F.P.S., F.L.S.	*W. J. Bush & Co. Ltd.*
G. I. Smales, B.Sc., F.R.I.C.	*Parke, Davis & Co.*
Miss G. M. Wells, B.Sc., A.P.I.	*Beecham Research Laboratories Ltd.*
A. J. Woodgate, B.Sc.	*Stafford Allen & Sons Ltd.*
Miss A. M. Parry Miss C. H. Tinker } (*Secretaries*)	

* Appointed May, 1962. † Resigned March, 1962.

TERMS OF REFERENCE—"To investigate methods of assay of capsicum and capsicum products with particular reference to the determination of the capsaicin content."

PROGRESS OF WORK—

Owing to the potential explosion hazards associated with the preparation of dry diazo-compounds, the Panel's work has been concentrated on perfecting an alternative colorimetric method of assay for the determination of capsaicin. This is based on the reaction of phenols with dichloroquinone-chloroimide (Gibb's reagent) to give a blue colour that can be evaluated spectrophotometrically. The Panel has also developed an assay procedure for determining capsaicin in Capsicum Wool B.P.C.

A report embodying these, together with certain amendments that have been found desirable to the originally published methods (*Analyst*, 1959, **84**, 603), is in preparation. With the completion of this report, the Panel's programme of work will come to an end.

PANEL 3: ANTHRAQUINONE DRUGS

CONSTITUTION—

W. Mitchell, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Stafford Allen & Sons Ltd.*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.	*University of London (Professor of Pharmacognosy)*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
Miss H. M. Perry, M.Sc., F.R.I.C.	*Stafford Allen & Sons Ltd.*
J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S.	*Bradford Institute of Technology (Department of Pharmacy)*
H. A. Ryan, B.Sc., F.R.I.C.	*Westminster Laboratories Ltd.*
W. Smith, B.Sc., F.R.I.C.*	*Allen & Hanburys Ltd.*
R. V. Swann, B.Sc., F.R.I.C.	*Allen & Hanburys Ltd.*
Miss A. M. Parry Miss C. H. Tinker } (*Secretaries*)	

* Resigned September, 1962.

TERMS OF REFERENCE—"To investigate methods for estimating the purgative activity of drugs and preparations of drugs containing anthraquinone derivatives with a view to recommending standard methods of assay."

PROGRESS OF WORK—

Since this Panel had been in suspension since May, 1960, no progress of work can be reported. As mentioned earlier in this Report, it resumed work in December, 1962, after its associated Panel (3A) on bioassay methods had submitted its report on a unified biological procedure for senna fruit that can be used as a form of "yardstick" for correlating chemical methods with purgative activity in mice.

Dr. Rowson, the Panel's original Chairman, resigned from that office in July, 1957, on his appointment to a post abroad, and Dr. Mitchell succeeded him. Now, on Dr. Rowson's return to this country, Dr. Mitchell has asked to be relieved of this duty, which he assumed on a temporary basis, and Dr. Rowson is once more the Chairman of the Panel. The Joint Committee thanks Dr. Mitchell for so ably stepping into the breach.

PANEL 3A: ANTHRAQUINONE DRUGS (BIOLOGICAL ASSAY)

CONSTITUTION—

K. L. Smith, M.P.S. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
P. F. D'Arcy, B.Pharm., Ph.D., M.P.S.*	*Allen & Hanburys Ltd.*
R. T. Brittain	*Allen & Hanburys Ltd.*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S.	*University of London (Professor of Pharmacognosy)*
G. A. Stewart, B.Sc., Ph.D., A.R.I.C.	*Wellcome Biological Control Laboratories*
Miss A. M. Parry Miss C. H. Tinker (*Secretaries*)	

* Resigned March, 1962.

TERMS OF REFERENCE—"To study biological methods for the assay of anthraquinone drugs."

PROGRESS OF WORK—

The reproducibility of results between laboratories for the biological assay method when mice and the same solutions and the same conditions were used has been examined. With this information, re-examination of two samples of senna pod (previously examined by Panel 3 by a chemical method) against a common sennoside standard has been made. The disagreement between laboratories beyond the limits of experimental error has been removed. A full report of these investigations was submitted to Panel 3, who made the original request for the work to be done.

PANEL 5: LONCHOCARPUS AND DERRIS

CONSTITUTION—

R. F. Phipers, B.Sc., Ph.D. (*Chairman*)	*Cooper Technical Bureau*
R. Buckley, B.Sc., A.R.I.C.	*Plant Protection Ltd.*
J. A. Dawson, B.Sc., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
W. E. Drinkwater, F.R.I.C.	*Boots Pure Drug Co. Ltd.*
R. V. Foster, M.Sc., A.R.I.C.	*Cooper Technical Bureau*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
J. T. Martin, D.Sc., F.R.I.C.	*University of Bristol (Long Ashton Research Station)*
R. A. Rabnott	*Analytical and Consulting Chemist*
F. H. Tresadern	*Stafford Allen & Sons Ltd.*
Miss A. M. Parry (*Secretary*)	

TERMS OF REFERENCE—"To investigate methods of assay of derris, lonchocarpus and their preparations, with particular reference to the determination of the rotenone content."

PROGRESS OF WORK—

The Panel continued its work on a colorimetric method for determining low concentrations of rotenone, as a routine control procedure. Optimum conditions have been established and details of the method are published in the Panel's second Report (*Analyst*, 1961, **86**, 748), but it is not intended to be applied as a standard method.

The Panel has now completed its programme of work and has been disbanded.

PANEL 6: PYRETHRUM

CONSTITUTION—

W. Mitchell, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Stafford Allen & Sons Ltd.*
E. A. Baum, D.Sc., I.C.N.*	*Pyrethrum Bureau*
H. E. Coomber, B.Sc.	*Mitchell Cotts & Co. Ltd.*
L. Donegan	*D.S.I.R., Tropical Products Institute*
M. Elliott, Ph.D.	*Rothamsted Experimental Station*
J. R. Furlong, O.B.E., Ph.D.†	*Pyrethrum Bureau*
A. D. Harford	*British Petroleum Research Centre*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
W. S. Manson, B.Sc., A.R.I.C.	*Cooper Technical Bureau*
R. A. Rabnott	*Analytical and Consulting Chemist*
F. H. Tresadern	*Stafford Allen & Sons Ltd.*
T. F. West, Ph.D., D.Sc., A.M.I.Chem.E., F.R.I.C.	*Society of Chemical Industry*
Miss A. M. Parry Miss C. H. Tinker (*Secretaries*)	

* Appointed May, 1962. † Resigned April, 1962.

TERMS OF REFERENCE—"To investigate methods of assay of pyrethrum flowers and pyrethrum extract with a view to recommending a standard chemical or physical method of assay."

PROGRESS OF WORK—

The Panel has continued its study of the mercury-reduction method. The variant of the method that has generally been used in this country was revised, and has given excellent inter-laboratory agreement of results in the hands of members of the Panel, this applying both to pyrethrum extracts and to pyrethrum flowers. Unfortunately, use of the revised version of the method by three commercial laboratories (each represented on the Panel) has not secured improved agreement between their routine results.

Independent work carried out in the laboratories of two companies represented on the Panel has shown that the Panel's variant of the mercury-reduction method records as "pyrethrin I" a significant amount of extraneous material. Several alternative procedures that appear partly to eliminate this interference have been proposed. Pending a fuller study of these, and other, possibilities, it has been decided to study the A.O.A.C. version of the mercury-reduction method, which appears to include less extraneous material.

A modified procedure (*J. Sci. Food Agric.*, 1955, **6**, 465) for the determination of "pyrethrin II" has been studied. Concurrently, it has been approved by the A.O.A.C. for inclusion in their published method.

PANEL 7: THYROID

CONSTITUTION—

C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
R. E. A. Drey, B.Sc., F.R.I.C.	*Wellcome Chemical Works*
Miss S. J. Patterson, B.Sc., A.R.I.C.	*D.S.I R., Laboratory of the Government Chemist*
R. L. Clements, B.Sc.*	*D.S.I.R., Laboratory of the Government Chemist*
N. A. Terry, B.Pharm., F.P.S.	*British Drug Houses Ltd.*
C. Vickers, B.Sc., A.R.I.C.	*Boots Pure Drug Co. Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed December, 1962.

TERMS OF REFERENCE—"To investigate the possibility of devising a chemical method for determining the pharmacologically active constituents of thyroid."

PROGRESS OF WORK—

During the first year of its work, the Panel has concentrated on a general investigation of the problems involved in order that it might assess the possibility of success. Work has been carried out on each of the three main sub-divisions of a possible assay, namely (*i*) hydrolysis of the thyroid, (*ii*) separation of the active iodinated amino acids by paper or thin-layer chromatography and (*iii*) determination of the separated fractions.

Stage (*i*), which is likely to prove the most difficult for quantitative work, has so far received only a minimum of attention. Both alkaline and enzymatic conditions of hydrolysis are being considered. Chromatographic separation of synthetic mixtures of iodinated amino acids, Stage (*ii*), has been successfully carried out by a number of different systems, and this work is now being extended to the qualitative examination of thyroid hydrolysates. For the determination of the very small amounts of iodine deriving from such separations, Stage (*iii*), several methods have been investigated. The ceric sulphate - arsenious oxide catalytic method is considered unsuitable for routine application to occasional samples, such as would be necessary for the commercial examination of thyroid. The use of ultraviolet absorption measurements (at 288 mμ) and the formation of a starch - iodine complex both show considerable promise, and these are being further investigated.

APPENDIX I

THE SOCIETY FOR ANALYTICAL CHEMISTRY ANALYTICAL METHODS TRUST

ACCOUNTS FOR THE YEAR ENDED OCTOBER 31ST, 1961

Income and Expenditure Account for the Year Ended October 31st, 1961

1960 £	£		£	£
	246	Rent, Light, Heat and Telephone	272	
	3497	Salaries	2969	
	46	Office Equipment ..	63	
	138	Printing and Stationery	358	
	23	Travelling Expenses ..	33	
	136	Expenses of Meetings ..	29	
	42	Audit Fee and Accountancy	21	
	101	Postage and Petty Expenses	123	
4229	—			3868
200		Scholarship Grant and Award for Research ..		1132
148		Contribution to Fittings and Decorations of Council Room		—
1706		Excess of Income over Expenditure for the year ended 31st October, 1961 transferred to Accumulated Fund		1673
£6283				£6673

1960 £	£		£
	4461	Subscriptions from Industry	5910
	955	Income Tax recovered on Covenanted Subscriptions for the years 1954/55 to 1958/59	—
5416	—		
	10	Interest from Investments (gross)	8
	521	Bank Deposit Interest ..	650
	336	Profit on Sales of "Recommended Methods for the Analysis of Trade Effluents" received from the Society for Analytical Chemistry	105
£6283			£6673

Accumulated Fund

1960 £		£
15,831	Balance carried to Balance Sheet	17,504
£15,831		£17,504

1960 £		£
14,086	Balance at October 31st, 1960 ..	15,831
1,706	Excess of Income over Expenditure for the year ended 31st October, 1961	1,673
39	Increase in Value on Redemption of £100 Government of Ceylon $3\frac{1}{2}\%$ Stock 1959	—
£15,831		£17,504

Balance Sheet at 31st October, 1961

1960 £		£
15,831	*Accumulated Fund*	17,504
2728	*Sundry Creditors*	1064
£18,559		£18,568

1960 £	£		£	£
		Investments (at Cost):		
	83	£100 3½% Conversion Stock ..	83	
	100	£100 3½% War Stock	100	
183				183
		(Market Value at 31.10.61, £105)		
36		*Sundry Debtors* ..		37
		Cash:		
		At Banks on—		
	11,000	Deposit Account		11,000
	7340	Current Account	7348	
18,340				18,348
£18,559				£18,568

Signed on behalf of the Analytical Methods Trust Fund G. H. LLOYD-JACOB, *Chairman*,
J. HUBERT HAMENCE, *Honorary Treasurer.*

Report of the Auditors to the Trustees of The Society for Analytical Chemistry Analytical Methods Trust Fund

We have examined the above Balance Sheet which in our opinion gives a true and fair view of the state of affairs of the Trust Fund at 31st October, 1961.

10, New Court,
Lincoln's Inn,
LONDON, W.C.2.

16th January, 1962.

(Signed) RIDLEY, HESLOP & SAINER
Chartered Accountants,
Auditors.

APPENDIX II

THE SOCIETY FOR ANALYTICAL CHEMISTRY ANALYTICAL METHODS TRUST ACCOUNTS FOR THE YEAR ENDED OCTOBER 31ST, 1962

Income and Expenditure Account for the Year Ended October 31st, 1962

1961 £	£		£	£
	272	Rent, Light, Heat and Telephone	334	
	2969	Salaries	2371	
	63	Office Equipment ..	13	
	358	Printing and Stationery	83	
	33	Travelling Expenses ..	30	
	29	Expenses of Meetings ..	41	
	21	Audit Fee and Accountancy	21	
	123	Postage and Petty Expenses	97	
3868				2990
1132		Scholarship Grant and Award for Research		-
1673		Excess of Income over Expenditure for the year ended 31st October, 1962 transferred to Accumulated Fund		1589
£6673				£4579

1961 £		£	£
5910	Subscriptions from Industry ..		3787
	Interest from Investments		
8	Gross	7	
—	Taxed (net)	52	
			59
650	Bank Deposit Interest		603
105	Profit on Sales of "Recommended Methods for the Analysis of Trade Effluents" received from the Society for Analytical Chemistry ..		130
£6673			£4579

Accumulated Fund

1961 £		£
17,504	Balance at 31st October, 1962 carried to Balance Sheet..	19,093
£17,504		£19,093

1961 £		£
15,831	Balance at October 31st 1961, ..	17,504
1673	Excess of Income over Expenditure for the year ended 31st October, 1962	1589
£17,504		£19,093

Balance Sheet at 31st October, 1962

1961 £	£		£	£
17,504		Accumulated Fund		19,093
		Sundry Creditors		
	21	Accountancy ..	21	
	1043	The Society for Analytical Chemistry	2924	
1064				2945
£18,568				£22,038

1961 £	£		£	£
		Investments (at Cost):		
	83	£100 3½% Conversion Stock ..	83	
	100	£100 3½% War Stock	100	
		350 Debenhams Ltd. 10s. Ordy Shares	747	
		£250 Imperial Chemical Industries Ltd. Ordy Stock ..	736	
		500 Philip Hill Investment Trust Ltd. 5s. Ordy Shares	708	
		£300 Renold Chains Ltd. Ordy Stock..	685	
		300 Royal Insurance Co. Ltd. 5s. Ordy Shares	735	
		400 Wharf Holdings Ltd. Ordy Shares	706	
183				4500
		(Market Value at 31.10.62, £4225)..		
37		*Sundry Debtors* ..		26
		Cash at Banks:		
	11,000	United Dominions Trust Ltd. on Deposit Account	11,000	
	7348	Barclays Bank Limited on Current Account ..	6512	
18,348				17,512
£18,568				£22,038

Signed on behalf of the Analytical Methods Trust Fund G. H. LLOYD-JACOB, *Chairman*,
J. HUBERT HAMENCE, *Honorary Treasurer.*

Report of the Auditors to the Trustees of The Society for Analytical Chemistry Analytical Methods Trust Fund

We have examined the above Balance Sheet which in our opinion gives a true and fair view of the state of affairs of the Trust Fund at 31st October, 1962 and is in accordance with the Books kept by the Trustees. We have verified the Investments and found them to be in order.

10 New Court,
Lincoln's Inn,
LONDON, W.C.2.
28th June, 1963.

(Signed) RIDLEY, HESLOP & SAINER
Chartered Accountants,
Auditors.

APPENDIX III

SUBSCRIBERS TO THE TRUST FUND DURING 1961 AND 1962

Albright & Wilson Ltd.
Alginate Industries Ltd.
Ashburton Chemical Works Ltd.
The Associated Octel Co. Ltd.
Associated Chemical Companies Ltd.
Associated Portland Cement Manufacturers Ltd.
Boots Pure Drug Co. Ltd.
The British Aluminium Co. Ltd.
The British Arkady Co. Ltd.
British Cod Liver Oils (Hull and Grimsby) Ltd.
The British Drug Houses Ltd.
British Oxygen Research & Development Ltd.
Cadbury Brothers Ltd.
Central Electricity Generating Board.
Cerebos Ltd.
Cooper, McDougall & Robertson Ltd.
Courtaulds Ltd.
Crosse & Blackwell (Holdings) Ltd.
Cyanamid of Great Britain Ltd.
The Distillers Co. Ltd.
Dunlop Rubber Co. Ltd.
Norman Evans & Rais Ltd.
Fisons Ltd.
A. Gallenkamp & Co. Ltd.
Glaxo Laboratories Ltd.
Goulding Fertilisers Ltd.
Arthur Guinness, Son & Co. (Park Royal) Ltd.
T. Hedley & Co. Ltd.
H. K. Heinz Co. Ltd.
E. M. Hobbs, Esq.
Hopkin & Williams Ltd.
Horlicks Ltd.
Hovis McDougall.
Huntley & Palmers Ltd.
Imperial Chemical Industries Ltd.
International Flavors & Fragrances (Great Britain) Ltd.
Johnson Matthey & Co. Ltd.
Kellogg Co. of Great Britain Ltd.
Kodak Ltd.
Laporte Chemicals Ltd.
Laporte Industries Ltd.
London County Council.
J. Lyons & Co. Ltd.
May & Baker Ltd.
Merck Sharp & Dohme Ltd.
The Metal Box Co. Ltd.
The Millers' Mutual Association
Monsanto Chemicals Ltd.
National Coal Board
Nestlé Co. Ltd.
Novadel Ltd.
Oxo Ltd.
Parke Davis & Co.
Pfizer Ltd.
The Pharmaceutical Society of Great Britain.
Procea Products Ltd.
The Pyrethrum Bureau
Quaker Oats Ltd.
Roche Products Ltd.
Rowntree & Co. Ltd.
J. Sainsbury Ltd.
Schweppes (Home) Ltd.
"Shell" Research Ltd.
Smith Kline & French Laboratories Ltd.
Stafford Allen & Sons Ltd.
John & E. Sturge Ltd.
Tate & Lyle Ltd.
Unilever Ltd.
Vitamins Ltd.
Wallace & Tiernan Ltd.
Washington Chemical Co. Ltd.
The Wellcome Foundation Ltd.
Weston Research Laboratories Ltd.
West Norfolk Farmers Manure & Chemical Co-operative Co. Ltd.
William R. Warner & Co. Ltd.
John Wyeth & Brother Ltd.

Beer's Law and its Use in Analysis

A Review

BY G. F. LOTHIAN

(*University of Exeter, Department of Physics, The Washington Singer Laboratories, Prince of Wales Road, Exeter*)

SUMMARY OF CONTENTS

Introduction: deduction of Beer's law
Terms and symbols
The validity of Beer's law:
  The use of monochromatic radiation: finite waveband and stray radiation
  Parallelism of beam
  Non-homogeneous specimens: chemical analysis by turbidimetric measurements, determination of particle size, absorption curves of granulated materials
  Molecular interactions
Determination of absorbing materials

IF a parallel beam of homogeneous (*i.e.*, one wavelength) radiation of intensity I falls on a sufficiently thin layer (thickness *dl*) of absorbing material, a small fraction will be absorbed. If the thickness of the layer is doubled, the fraction absorbed will be doubled; this is obvious if successive layers behave independently of one another (no multiple scattering) and if each thin layer is so sparsely populated with absorbing molecules that they do not hide behind one another in the path of the beam. Thus for thin layers the fraction absorbed is proportional to the layer thickness, *i.e.*—

$$dI/I = -\mu dl \qquad \text{(1)}$$

where μ is a constant known as the absorption coefficient. (The minus sign occurs because an increase in path means a decrease in intensity). Integration of this expression gives

$$\log_e I_0/I = \mu l \qquad \text{(2)}$$

where I_0 is the incident intensity at $l = 0$. This equation may be rewritten in various ways—

$$\begin{aligned}\log_{10} I_0/I &= 0{\cdot}4343 \log_e I_0/I \\ &= 0{\cdot}4343\, \mu l \\ &= Kl \\ &= d \qquad \text{(3)}\end{aligned}$$

where K is a new constant, or—

$$t = I/I_0 = e^{-\mu l} \text{ or } 10^{-Kl} \qquad \text{(4)}$$

where t is the fraction transmitted.

Any of these forms expresses the law first formulated about 1730 by Lambert and by Bouguer.

If the concentration, *c*, of absorbing molecules is doubled and the path-length halved so that the total number remains the same, the absorption (d or t) will remain the same, provided that the molecules do not come so close together that they come within each other's influence, modifying the energy levels or even causing chemical changes. That is, the absorption will be a function of the total number of molecules, *i.e.*, of the product *cl*, and equation (3) may be rewritten—

$$d = \log_{10} I_0/I = Ecl \text{ or } \epsilon cl \qquad \text{(5)}$$

E, ϵ are new constants, the latter symbol being used when concentration *c* is in gram-molecules per litre.

This last equation is Beer's law, first stated in 1852[1]; it includes the earlier Lambert's law. In words it may be summarised by saying that the absorption of radiation by molecules depends only on their total number. Its original formulation has been recently discussed by Pfeiffer and Liebhafsky.[2]

For a mixture of several different absorbing substances the above argument may be repeated—provided again that the mixture is sufficiently dilute—when it may be shown that the optical densities of the several components are added, *i.e.*—

$$d = (E_1c_1 + E_2c_2 + - - -)l \quad .. \quad .. \quad .. \quad .. \quad (6)$$

This is the generalised form of Beer's law used in analysis of mixtures.

TERMS AND SYMBOLS

d, K, E and ϵ (equations 3 and 5) are fundamental in the use of Beer's law. Their usefulness is obvious, since the value of d is proportional to concentration and path length, and the value of K is proportional to concentration. The names of these quantities are summarised, together with the definitions, in Table I. In 1942 a report in *The Analyst*[3] recommended the use of names shown in column 1 and of the symbols shown in column 4. At later dates some alternative names shown in columns 2 and 3 were recommended[4,5,6] in the U.S.A. and are now commonly used there. The absorption coefficient, μ, is in common use for absorption of x radiation, but not for optical radiations.

TABLE I

TERMS, SYMBOLS AND DEFINITIONS

Term				
Society of Public Analysts[3]*	Brode[4] and Hughes[5]	National Bureau of Standards[6]	Symbol	Definition
Optical density (or extinction)	Absorbance	Absorbance	d†	$\log_{10}(I_0/I)$—logarithm of reciprocal transmission
Extinction coefficient	—	Absorbance index	K	d/l—optical density (etc.) per unit path length
Specific extinction coefficient	Absorptivity	Absorbancy index	E	d/cl—optical density for unit path length and concentration
Molecular extinction coefficient	Molar absorptivity	Molar absorbancy index	ϵ	d/cl—specific extinction coefficient (etc.) for concentration of 1 gram-mole per litre

* Now The Society for Analytical Chemistry.

† D, E or **E** sometimes used.

THE VALIDITY OF BEER'S LAW

If a measurement of optical density, d, is made, with known path length l, then the concentration, c, can be calculated from equation 5 if the specific extinction coefficient (E or ϵ) is known. But before this equation can be used it is necessary to test that the equation holds under the particular conditions of a measurement. If it is found not to hold, it is possible to establish an empirical relation (calibration curve) between optical density and concentration. But it is dangerous to do this blindly; unless one knows why Beer's law is not holding, one may not know within what limits the empirical relation may be expected to apply. It is therefore most important to consider in detail reasons for departure from Beer's law.

It will be appreciated from the introductory paragraphs that Beer's law may be expected to apply for—

(1) perfectly monochromatic radiation

(2) travelling in an optically homogeneous medium (no scattering of radiation)

(3) as a strictly parallel beam.

(4) It is further necessary that the absorbing molecules are never close enough to one another or to other (impurity) molecules that the molecule structure and hence the energy levels are affected.

These four conditions are ideals to which it is possible only to approximate. The degrees of approximation necessary and the effects that appear when the limits are exceeded will now be discussed for each of these conditions in turn.

THE USE OF MONOCHROMATIC RADIATION—

The constant E (or ϵ) varies with wavelength so that, if the incident radiation contains a number of wavelengths, the wavelength distribution of the radiation reaching the deeper layers of an absorbing specimen will be modified and the conditions assumed in equation (1), that all elementary layers absorb the same fraction of radiation, is no longer true. In practice, radiation can never be perfectly monochromatic and will depart from this condition in two respects—

(*i*) Finite wavebands. It will include a continuous band of radiation extending over a finite waveband centred about the required wavelength.

(*ii*) Scattered radiation. Radiation of widely different wavelengths from the desired wavelength arising from scatter, unwanted reflections, etc., in a monochromator will also form part of the measuring beam. These two factors will be considered in turn.

Finite waveband—Fig. 1 shows spectrophotometric curves for two concentrations (ratio 4 to 1) of the same substance. If, in endeavouring to measure the optical density at the narrow long-wave peak, the "monochromatic" radiation used covers the waveband cd, it is obvious that the observed optical density will be less than the true value for the peak.

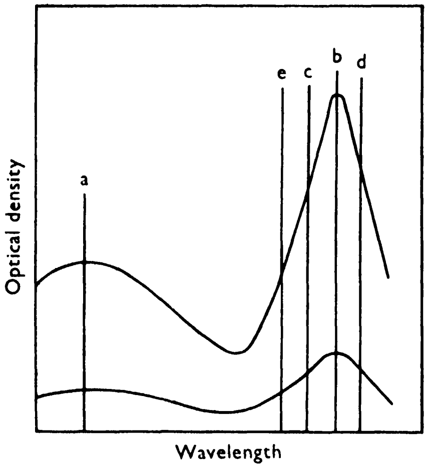

Fig. 1. Typical absorption curve with broad and narrow peaks

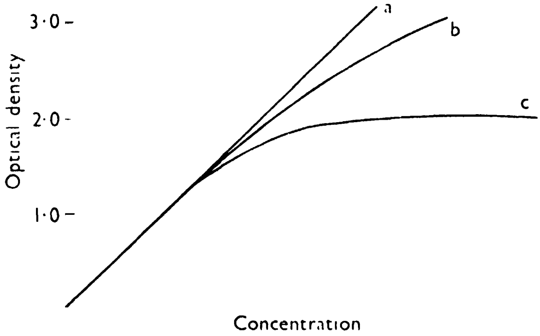

Fig. 2. Relation between optical density and concentration:

(*a*) Beer's law;

(*b*) Failure of Beer's law owing to finite waveband;

(*c*) Failure of Beer's law owing to stray radiation. Drawn to scale for 1 per cent. of stray radiation that is unabsorbed by the specimen

The difference between the observed and true maximum value will be proportionately greater for the higher concentration, from which it follows that the relation between optical density and concentration will no longer be linear, but curved as in b of Fig. 2. Calculations by Brodersen[7] have shown that even if measured optical densities are found to be proportional to concentration (curve a of Fig. 2) the observed values may be less than the "true" values for perfectly monochromatic radiation situated at the wavelength of the absorption maximum. (The difference amounted to 8 per cent. in a case calculated by him.)

It follows from all this that a spectrophotometric curve obtained with a monochromator passing a finite waveband will have its maxima depressed and its minima raised. The "true" curve can be estimated approximately from an observed curve by a graphical method first given by Paschen in 1897 and more recently explained by Slater[8] and also given in various text-books on spectrophotometry. Of course no treatment of observations can reveal detailed structure within the waveband used for the measurements.

The maximum waveband permissible, if Beer's law is to hold, thus depends on the measurements being made. Conditions are less stringent at a broad peak (a of Fig. 1) than at the narrow peak b of this figure; the former is obviously more satisfactory to use for analytical purposes. In atomic-absorption spectrophotometry, the width of an absorption line may be about 0·02 Å, and the source of radiation has to be a hollow-cathode discharge giving emission linewidths appreciably less than this. For measurements made at the peak of the α band ($\lambda = 5770$ Å) of oxyhaemoglobin, a bandwidth of 2 to 3 Å would be desirable—this is not easy to attain with a monochromator and a source giving a continuous spectrum, and it is preferable in determining haemoglobin to avoid this narrow absorption band. But with measurements on potassium chromate at the broad maximum at $\lambda = 3720$ Å, a bandwidth of 50 Å will leave Beer's law valid within about 1 per cent. It is obvious from Fig. 1 that the higher the concentration the sharper a maximum becomes, and efforts to increase precision by working at high optical densities (differential spectrophotometry) must mean a narrower waveband if Beer's law is to be maintained.

Stray radiation—In general a monochromator passes, in addition to an intensity I_0 of radiation of the required wavelength, an intensity S of stray radiation of other wavelengths. If a specimen whose optical density is being measured transmits this stray radiation without any absorption, the apparent or measured value will be

$$d_{\text{appt}} = \log_{10} \frac{I_0 + S}{I + S}$$

in place of the value given by equation (5). As the concentration approaches infinity, when according to Beer's law d should approach infinity, the apparent optical density value will now approach a maximum value of

$$d_{\text{appt}} = \log_{10} \frac{I_0 + S}{S}$$

and a curve of optical density against concentration will now be as c in Fig. 2. If the stray radiation is 1 per cent. of I_0, the maximum value of apparent optical density will be 2·0.

Departure from Beer's law from this cause can be a frequent source of error. It is dangerous to use empirical calibration curves such as c of Fig. 2. This is partly because the curve may change from day to day with changing instrumental conditions (changing values of S due to dust, tarnished surfaces, etc.). But, in addition, the measurement will be affected by the presence of impurities; to take an extreme instance, if a specimen being measured contains, unknown to the operator, impurities completely transparent at the working wavelength, but which completely absorb the stray radiation of other wavelengths, the calibration curve c of Fig. 2 will be replaced by the straight line a—and the operator will not know this!

Tests for the effective presence of stray radiation may be made in one of several ways—

(*a*) By making measurements to construct a calibration curve as in Fig. 2 under conditions where one knows that Beer's law ought to be applicable. Some of the first workers to describe the use of this method were Hogness, Zscheile and Sidwell[9] who found, using their own instrument at $\lambda = 3700$ Å with a hydrogen-discharge lamp, a value for S/I_0 equal to 0·004. A modified form of this method is to insert in the beam a specimen having a narrow absorption band at such a thickness that one would expect zero transmission, and then to measure the resulting intensity of radiation or the optical density of the specimen.

(*b*) An alternative method, complementary to the first, is to endeavour to reduce the stray radiation by inserting a filter having high transmission at the required wavelength and low transmission at the wavelengths at which stray radiation might be expected. With a double-beam instrument the filter should, of course, be inserted into both beams. If the use of such a filter then brings about a change in the measured value of optical density, the presence of stray radiation is to be inferred, and it would be desirable to retain the added filter for measurements under these conditions.

(*c*) Pritchard[10] has described how to determine the stray radiation of various wavelengths for various wavelength settings, by passing pure radiation from a double monochromator into the spectrophotometer.

The effective amount of stray radiation is likely to be great (S/I_0 large) when the effective value of I_0 is small. This will be most likely to occur when working at wavelengths near the limits of usefulness of the radiation source, the optical system or the detector. For example, a tungsten-filament lamp *can* be used at $\lambda = 3500$ Å, but the energy here is so small compared with the emission at longer wavelengths that there is grave risk of measurements of optical density being grossly in error. Thus a specimen whose absorption is small throughout the visible spectrum and continuously increasing in going to shorter wavelengths in the ultraviolet, may well show a completely spurious absorption maximum at, say, $\lambda = 3700$ Å; a smaller apparent optical density at $\lambda = 3500$ Å being measured because the energy here may be mostly visible radiation. Similar difficulties occur in the far infrared, where the emission of one of the usual incandescent sources is very small compared with the emission in the region $\lambda = 1$ to $3\,\mu$. Likewise, measurements with a quartz optical system near its useful limit of $\lambda = 2000$ Å, or a photomultiplier near its long wave limit in the red, need careful consideration before being passed as valid. This is not the place to discuss how to reduce the amount of stray radiation; but the examples mentioned are intended to emphasise the need to test for Beer's law under the exact conditions used in subsequent measurements—in particular: nature of specimen, source of radiation, slit width, dispersing system, radiation detector and alignment of components. Goldring, Hawes, Hare, Beckman and Stickney[11] have published some curves showing the effects of stray radiation on measurements of potassium chromate; in their paper they discuss this and other sources of error in some detail, and include a useful bibliography of 19 references.

PARALLELISM OF BEAM—

In the introduction we have presupposed a parallel beam—an ideal that can never be obtained. A perfectly parallel beam must come from a point source and can carry only an infinitesimal amount of energy. In practice a beam of finite angular size (see Fig. 3) must be used. For a beam passing through a specimen at an angle θ to the axis the path length and hence the optical density will be increased by a factor $1/\cos\theta$. If the extreme value of θ through the specimen is 9°, the optical density will be increased by 1 per cent. ($1/\cos 9° = 1{\cdot}01$). If the refractive index of the specimen is 4/3, the corresponding angle outside the specimen will be $\theta = 12°$; this is greater than the semi-angle of beam in most commercial spectrophotometers, so that the finite angle will not in general be a source of error. But if efforts are made to increase precision by working at high optical densities (say $d = 3$) to a precision of 0·005, as is sometimes done in differential spectrophotometry, the finite angular size of beam may well cause departure from Beer's law. The effect is analogous to that arising from a finite waveband, but is complementary to the latter—the central axial beam giving a minimum optical density.

As will be seen in the next section, the angular size of beam is more important when scattering specimens are being measured.

NON-HOMOGENEOUS SPECIMENS—SCATTERING IN THE SPECIMEN—

A specimen may be fortuitously turbid; *i.e.*, it may include some suspended or colloidal material as an impurity in what would ideally be a homogeneous solution. Provided the turbidity is small, the optical densities due to absorption and scattering are additive, and it is often possible to determine the latter separately by extrapolation of measurements at adjacent wavelengths where the absorption is zero.

Much more must be said about specimens that inherently scatter radiation—colloidal solutions and suspensions, where it is the light scattering material in which one is interested. Such measurements may be made for various purposes—

(1) for chemical analysis—as in the determination of sulphate as barium sulphate,

(2) as a means of determining particle size,

(3) to determine the actual absorption of the material forming (part of) the particles —*e.g.*, the absorption of powdered materials incorporated in a pressed disc of potassium bromide or the absorption of pigment in plant or animal cells.

The argument used in the opening paragraphs to deduce Beer's law may be repeated for a suspension or colloid simply by speaking of "particles" instead of molecules. One assumes that some or all of the radiation incident on a particle is lost from the transmitted beam—by absorption or by scattering or refraction into another direction. The fraction so lost depends in a complicated way on a number of factors; the matter can only be summarised here by saying that for a perfectly parallel beam the obscuring power of a single particle is not equal to its projected area A, as might be expected from geometrical optics, but to KA, where K (quite distinct from the K in Table I) is often known as the scattering area coefficient. The value of K (somewhere between 0 and 5) is a function of—

(*i*) particle size relative to wavelength;
(*ii*) particle shape and orientation in the beam;
(*iii*) refractive index of the particle relative to the surrounding medium;
(*iv*) the true absorption of the particle.

In practice the angles θ_s and θ_d of the incident and measured beams must be finite (see Fig. 3) so that some of the scattered radiation will be picked up by the detector, reducing the effective opacity; *i.e.*, reducing the coefficient K. Thus K is also a function of—

(*v*) the beam angles θ_s and θ_d.

If all these five conditions remain constant so that K remains constant, Beer's law may be expected to hold provided—

(1) The beam angles θ_s and θ_d of Fig. 3 are so small that the path lengths are not significantly different for the various beams through the specimen—as already discussed for homogeneous specimens.

(2) Secondary scatter may be neglected. Radiation scattered out of the observed beam may suffer secondary scatter back into the observed beam. This secondary scatter will be more pronounced at high optical densities, reducing their value and giving an optical-density - concentration curve as in b of Fig. 2. The larger the angles θ_s and θ_d the lower the optical density at which such failure of Beer's law will set in.

The effects of these considerations on measurements made for the three different purposes listed on p. 682 are discussed below in turn.

Chemical analysis by turbidimetric measurements—In the determination of barium sulphate, Treon and Crutchfield,[11] for example, found Beer's law to hold for the largest optical densities they used (up to 1·0) at $\lambda = 5700$ Å. But this conclusion would be useful only under the conditions of their work—type of spectrophotometer and its adjustment and the exact technique they used in preparing their suspensions. It is not really possible to be sure of repeating the technique exactly from day to day, and it is essential that all measurements be comparative—an unknown concentration being measured by comparison with known concentrations not very different from the unknown, the two suspensions being prepared side by side at the same time. Working in this way, one does not rely on the applicability of Beer's law except for interpolation over a small range.

Determination of particle size—For this sort of work it is desirable to have the angles θ_s and θ_d of the incident and emergent beams as small as possible in order to approximate to the conditions ($\theta_s = \theta_d = 0$) under which the scattering properties have been calculated.[13]

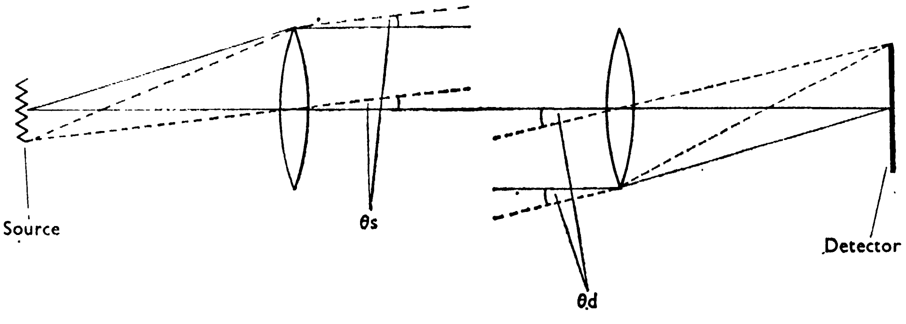

Fig. 3. Illustration of the finite angular sizes of the beam incident on a specimen—semi-angle θ_s—and of the beam received by the detector—semi-angle θ_d

Goulden[14] has described modifications of commercial instruments with this end in view and has made measurements to determine the size of the fat globules in milk. As explained above, such small angles help to maintain Beer's law to higher values of optical density. It is of course important to check by measurements at several concentrations or cell lengths that Beer's law is holding under the conditions of measurement. If secondary scatter is affecting the measurements they cannot, without correction, be used to calculate particle size.

Determination of absorption curves of granulated materials—More or less successful attempts have been made to deduce true absorption curves from transmission measurements on granulated materials; for example, haemoglobin in whole blood cells[15,16] and chlorophyll in cells of the alga, Chlorella.[17] But the theory developed so far involves some approximations, and at present it must be said that in general it is not safe to assume that absorption curves obtained from such measurements give the true curve that would be obtained from a homogeneous solution of the absorbing compound.

If the measurements are to be a function of the absorption only and not of the scattering properties, it is necessary to include all the scattered radiation in the measurement. Ideally this may be done by surrounding the specimen with an integrating sphere. But the theory and practice of this arrangement are complicated, and the method is not in general use. A good approximation is to use a translucent diffusing surface at the exit face of the specimen cell to direct a representative sample of the forward scattered radiation into the detector.

With these conditions of observation Beer's law may be expected to hold, but only in the limited sense that optical density will be proportional to the number density (number per unit area) of a given type of particle provided this is small enough for secondary scatter not to come into play. The optical densities will not be proportional to the values that would be obtained for the same amount of material uniformly dispersed in a homogeneous solution. This is because of the "sieve effect." Some of the beam of radiation passes outside the absorbing particles to reach the detector unabsorbed. This means that for a given number of particles the optical density will not exceed a limiting value, however great the absorption in each particle; hence absorption peaks will be depressed. It may be shown[18] that this effect disappears as the absorption of a single particle approaches zero. A consequence of this is that powdered materials (as in the potassium bromide pressed-disc method) should be ground as fine as possible. In addition, peaks may be slightly shifted because of refractive index changes near an intense absorption band. And if not enough of the scattered radiation is picked up, an absorption peak may well disappear altogether when a pigment is localised in cells.[16,19]

MOLECULAR INTERACTIONS—

When the concentration is sufficiently increased, an absorbing molecule can no longer be regarded as being isolated from other similar molecules by empty space (gas samples) or by an infinite ocean of solvent molecules. The energy levels, and hence the absorption spectrum, will become modified when molecules are in sufficient concentration to influence one another and consequently to reduce the influence of surrounding solvent molecules. In the classical language of chemistry this modification is explained as a chemical change that may be described as a change of polymerisation, isomerisation, ionisation, hydrolysis, etc. These effects form too vast a subject to be dealt with in this review and are appropriate to reviews on these individual chemical topics. It must suffice to say that, if a lack of proportionality of optical density and concentration cannot be ascribed to any of the causes already discussed in some detail, a chemical change must be considered.

A short mention should, however, be made of the case of absorption in a gas under changing pressure. As the pressure of a gas is increased, an absorption band is in general broadened with the result that the specific extinction coefficient at the centre of the band is depressed, *i.e.*, Beer's law is no longer valid. But it is found that even when this is so, the total area under an absorption band ($A = \int K d\nu$ or $\int \mu d\nu$ when the integration is carried out over the whole range of frequencies, ν, covered by the band) is often approximately proportional to the total concentration of absorbing molecules. This may be regarded as a modified version of Beer's law. Some spectrophotometers provide mechanical integrating attachments for determining this integral. The use of the area under an absorption curve for analytical work has been discussed in several original papers; see, for example, Oswald,[20] Ramsay[21] and Mills and Thompson.[22] These last workers show how, even when the spectrometer will not completely resolve a band, a correct value for A can be obtained by extrapolation to zero partial pressure by measurements on mixtures with an inert non-absorbing gas.

DETERMINATION OF ABSORBING MATERIALS

The use of Beer's law for determining a single absorbing component (equation 5) and of mixtures of several such components (equation 6) is common practice and is well covered in the various text-books on spectrophotometry, and so needs little consideration here.

As already explained, it is desirable to make measurements at the peaks of broad bands for several reasons: (*a*) At a *peak* so that the sensitivity is a maximum and interfering effects of absorption due to impurities is a minimum, and further so that a small error in wavelength setting will cause minimum change in optical density—measurement on the steep slope of a band (e in Fig. 1) is bad practice. (*b*) As already explained the use of a *broad* peak reduces troubles due to finite waveband.

For measurements on mixtures of *n* components, *n* measurements at *n* different wavelengths are needed to solve *n* equations of the type of equation (6). The wavelengths need to be chosen for maximum sensitivity—at each of the *n* wavelengths one component having a large absorption and the others having small absorption. Simplified methods of solution are given in various text-books.

The range of optical density in which a measurement is made can be adjusted by suitable choice of cell lengths. Much has been written about the optimum value of optical density to choose. For photographic and visual spectrophotometry a high value is, in general, the best. Various tests show that, with simple assumptions about the limiting sensitivity of a photo-electric or thermo-electric detector, the optimum precision is attainable at an optical density of 0·43. But it has also been shown[23] that this conclusion is modified by taking account of the fact that a measurement of optical density involves several operations—including setting a galvanometer or similar scale with no radiation falling on the detector, then with a blank cell (solvent) in the beam and finally with the absorbing specimen in the beam. These considerations may need to be further modified by reason of instability in the radiation source (voltage fluctuations). It is my opinion that the optimum optical density depends on so many factors—voltage stability and level of photo-electric (or thermo-electric) current—that an optimum value of d cannot be usefully calculated, but must be determined in any particular investigation by trial and error determination of repeatability of observations.

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The Application of Flying-spot Scanning to Particle Size Analysis in the Formulation of Pesticides*

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Pesticides are usually applied as dusts or sprays in which particle size is one factor that controls the impaction on and coverage of the target surface and the amount of drift of toxic material away from the target. Most pesticide sprays are formulated as emulsions, or suspensions of solid particles in water; the particulate size of these dispersions is important in determining both their relative stability and the biological toxicity of the deposits they produce on the target surface.

Particle size analysis in the pesticide field necessitates the study of a wide variety of particulate matter under many different conditions, and it has been found possible to use the "flying spot" automatic scanning technique to cover many of the problems involved. The advantages and disadvantages of this automatic method of particle sizing are discussed with particular reference to the sampling requirements to obtain the greatest accuracy in the results.

ONE of the essential functions of formulation is to make possible the distribution of extremely small amounts of pesticide over large surface areas. The most obvious way of achieving this is to reduce the particle size of the pesticide so that its surface area approaches that of the target surface. It is impossible to do this to the toxicant alone, but, if its bulk can be extended by dilution with biologically inert solids or liquids and this larger amount is applied in the form of extremely fine particles, a satisfactory distribution of pesticide becomes possible. Thus, the usual ways in which pesticides are applied are as sprays or as dusts. Aqueous sprays are usually preferred when reasonable amounts of water are available, but few pesticides are soluble in water, and so they have to be formulated as emulsions or as suspensions of solid particles. The formulation and application of pesticides is concerned, therefore, with the dispersion of particulate material in various ways, the most important of which are solid particles in air (dusts), oil droplets in water (emulsions), solid particles in water ("wettable powders") and sprays of these last two dispersed in air. A control over the particle size in all these types of dispersions may be important in determining the biological efficiency of the pesticide application.

SPRAYS—

The droplet spectrum formed when a liquid is sprayed into air can vary considerably with the spray equipment both in terms of the actual size of the drops and in the range of drop size found in the spray cloud. Both these factors may influence spray performance in several ways. The effect of droplet size on coverage of the target surface is illustrated in Fig. 1. This shows the area (in acres) covered by 10 and 50 gallons of an ideal spray (when all the droplets are the same size), the coverage being calculated on the assumption that each droplet will impact and spread to cover a circle whose diameter is 4 times that of the original droplet. A spread factor of this magnitude is an average figure for aqueous sprays containing a reasonable wetting agent on foliage. For many pests, good coverage of the target surface is essential for satisfactory control, but the total leaf area of an acre of crop may be anything from 1 to 30 acres. To provide a spray coverage equivalent to the crop area, either extremely large volumes of spray must be used or the spray must be composed of extremely small droplets. The modern tendency is to apply smaller and smaller volumes of spray per unit area, and between 10 and 50 gallons to the acre is common; a drop size of less than 100 μ is therefore desirable.

However, the process of spraying is not as simple as this, because, if small droplets are used, drift of the droplets away from the target may become serious. Fig. 2 shows the

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* Presented at the meeting of the Society on Wednesday, February 6th, 1963.

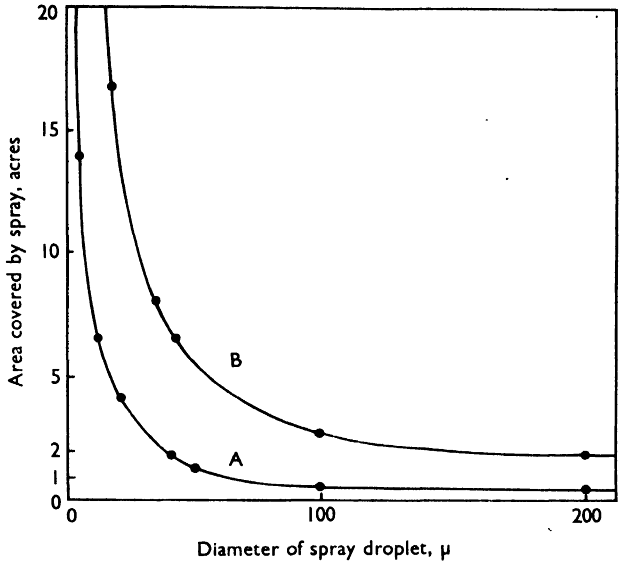

Fig. 1. Area covered by spray, assuming a spread factor of 4 × diameter of drop: curve A, 10 gallons; curve B, 50 gallons

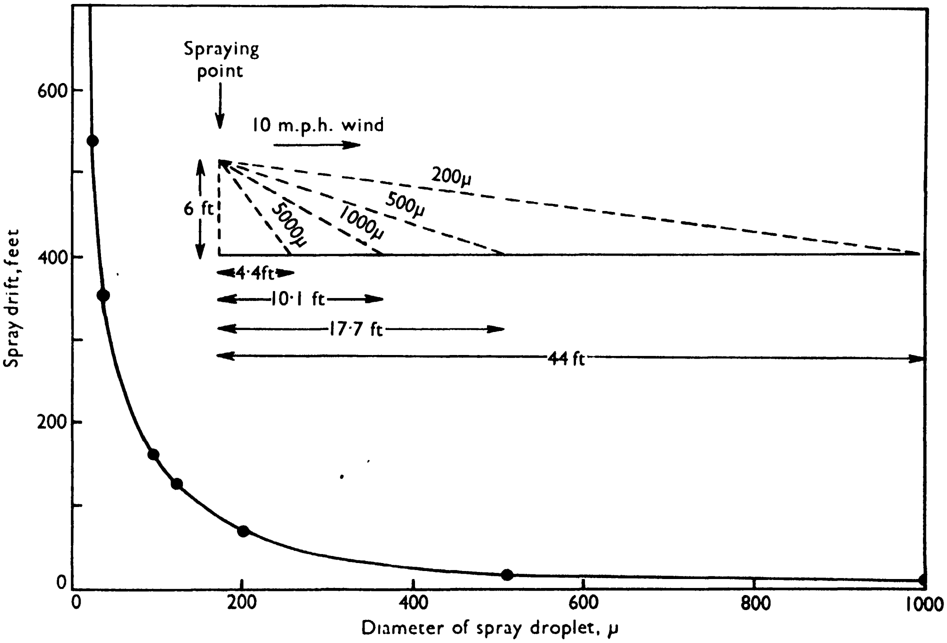

Fig. 2. Influence of drop size on spray drift

variation of spray drift with droplet size, the spray droplets being assumed to be initially stationary at 6 feet above ground level in a 10 m.p.h. wind. The spray drift is shown by the horizontal distance that the droplets travel before reaching the ground. In practice, 10 m.p.h. is about the maximum wind speed in which spraying would be carried out, but under these circumstances drops as large as 200 μ may drift a considerable distance.

The droplet size is one of the factors that influence the impaction efficiency of spray droplets and the retention of liquid on sprayed surfaces. It is impossible to generalise on the optimum drop size required for impaction on agricultural spray targets, because the latter vary so considerably in size, surface characteristics, degree of packing and in their movement relative to the spray droplets. The larger the drop size, the more readily will the drops impact on foliage, so that if it is desired that the spray should penetrate through thick foliage or dense undergrowth, then extremely small drop sizes are required.

The droplet size also governs the toxicity of a spray to flying insects. Work on the effects on flying mosquitoes of droplets of lubricating oil containing 8 per cent. of DDT shows that the maximum activity per unit weight of spray is found with a droplet size just over 10 μ; drop sizes either larger or smaller show much reduced activity.[1] A similar drop size - toxicity effect is found with many other flying pests, and this is important in the formulation of the aerosol generators that are now so widely used. Such formulations consist of a liquefied propellent gas, mixed with a solution of the toxicant in a non-volatile oil and held under pressure in a metal container. On releasing the pressure, the propellent gas vaporises and produces extremely fine droplets of the non-volatile oil; the droplet size is controlled by the proportion of propellent to oil and by the dimensions of the nozzle through which it is released.

DUSTS—

These have not, so far, been studied as thoroughly as sprays, but similar considerations apply in respect to drift and coverage of target. Dusts for foliage application would normally have a particle size between 50 and 70 μ, and so drift is again a serious problem unless the dusts are applied under still or extremely light wind conditions. One important factor in the application of dusts is the relative size of toxicant particles to filler particles. If considerable differences in size exist, toxicant and filler may settle out of the dust cloud in different areas and thus produce a patchy cover of toxicant.

The size of the particles in a dust cloud has a considerable effect on their impaction on foliage, their adhesion and on their resistance to weathering. Generally, impaction is improved as the particle size is increased, but resistance to weathering (by wind or rain) is decreased.

EMULSIONS AND SUSPENSIONS—

Most pesticides are formulated as water-dispersible oils (usually called emulsifiable concentrates) or as water-dispersible powders (wettable powders). The essential requirements in a wettable powder are that it should flow freely, even after prolonged storage, and that it should suspend well in water. The particle size can affect both these properties, and it is also one of the factors that determine the ease with which the toxicant is deposited on to the sprayed surface once the spray has impacted. The biological efficiency of the toxicant deposit is also governed by its particle size; in general, the smaller the particles, the better the results.

Similar considerations apply to the size of the oil globules in oil-in-water emulsion sprays.

In studying emulsion behaviour, it is valuable to be able to measure the rate of coalescence of emulsion globules in the dilute emulsion systems used in practice. This rate of coalescence is related to the ease of formation of the emulsion, its stability on standing and its behaviour after impaction on a plant surface. In a similar way, it is interesting to measure the rate of aggregation of solid particles in suspensions of pesticides.

PARTICLE SIZE ANALYSIS

Research into the formulation of pesticides necessitates the study of an extremely diverse range of particulate systems. Several methods are available for determining particle size,[2] but most of these methods are limited to one type of particulate system, *e.g.*, dusts in air or solid particles suspended in water. Many of these methods are used in studying specific formulation problems, *e.g.*, the over-all stability of emulsions and suspensions is usually estimated by fractional decantation methods. These have the advantage that the results can be directly related to the extent to which the dispersed phase will settle-out in the spray tank. Several other sedimentation methods are equally satisfactory for studying this type of system, but such methods are not generally applicable to all the particulate systems involved in the formulation and application of pesticides. For example, the measurement of spray droplet size can be satisfactorily carried out only by collecting the droplets on a suitable surface and sizing them. This method, in which the sample is placed under a microscope and the particles are compared by means of a suitable eye-piece graticule, has been used to a considerable extent, but it is really too tedious and slow to be satisfactory except in those instances when few samples have to be measured or when the particle size is reasonably uniform. Even then the method tends to be inaccurate because of the eye-strain that is involved.[3],[4]

These difficulties can be overcome by using an automatic method of counting and sizing the samples, and, if such methods are sufficiently versatile, they may be used for many of the particulate studies besides spray droplet sizing that are important in the formulation of pesticides. The flying-spot system[5,6] of scanning the sample was selected as being the most versatile method, with an instrument based on the flying-spot microscope and developed by Messrs. Rank-Cintel Ltd.

FLYING-SPOT PARTICLE RESOLVER—

The operation of the flying-spot particle resolver is shown diagrammatically in Fig. 3. A 700-line scanning raster, produced on the face of the scanning tube, is passed into the optical system. For convenience, this usually consists of a standard optical microscope, but, if lower magnifications are desired, it can be replaced by a simple projection lens. The image of the scan raster is focused on the sample to be examined, the scanned area decreasing as the magnification increases. The amount of light passing through the sample, when transparent backgrounds are used, or reflected from the sample if it is opaque, varies according to the optical density and configuration of the objects on the sample; these changes in light intensity are detected by a multiplier photocell, in which they are converted into an electrical signal. This signal is fed to the video amplifier, the output of which modulates the monitor cathode-ray tube, and thus a magnified image of the sample is produced on the monitor screen.

Automatic counting of all the particles within the scanned field is carried out by using the extra units shown in Fig. 3. If only one particle is on the sample slide when this is scanned by the flying spot, an intercept pulse will be produced which passes to the quantizer. This unit will pass on the pulse only if it is greater than a predetermined voltage level; it thus prevents counts from spurious particles and random noise. From here the pulse passes to a magnetic memory system (the one-line delay), the sum circuit and the pulse-forming unit where it is suitably shaped. After shaping, the pulse passes through an electronic gate, which is normally open, and then operates a dekatron counter tube. Acceptance of a count pulse is shown on the monitor screen by a bright spot appearing on the image of the particle. If this particle is larger than one picture element, *i.e.*, greater than the distance between two adjacent scanning lines, the flying spot will scan the same particle for a second time on its next scan line. A similar sequence of events occurs, but this time the sum circuit receives the new pulse from the quantizer plus the pulse from the previous scanning line, which is

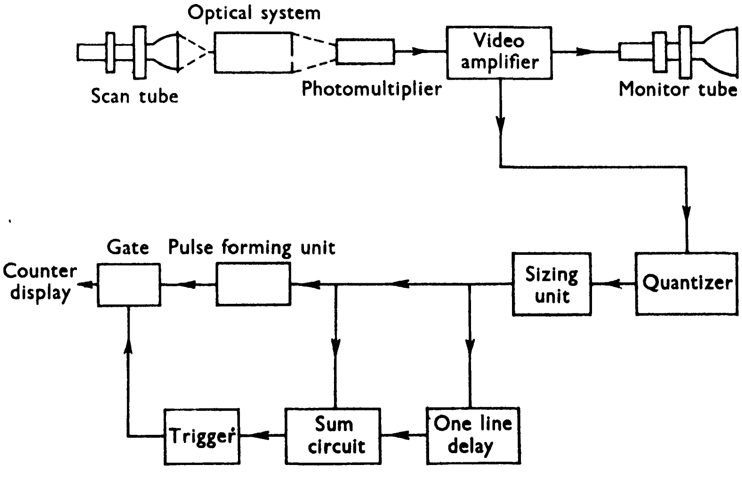

Fig. 3. Flying-spot particle resolver

released from the one-line delay unit. This state of coincident inputs produces an inhibiting pulse that shuts the gate and prevents a second count being recorded for the same particle.

Thus, in any given field every particle, however large, will be counted only once. It should be noted that this is an ideal situation and that, should the profile of the particle present a re-entrant along the line of the scan, two counts will be recorded from the same particle. Also, aggregates or particles that are not separated by more than two picture elements may be counted falsely.

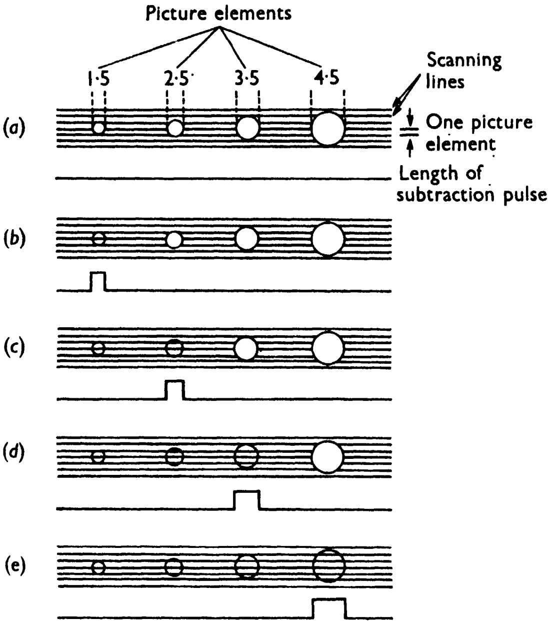

Fig. 4. Operation of sizing unit:

(*a*) Sizing unit set on O; no subtraction pulse is produced and therefore all four particles are counted.

(*b*) Sizing unit set on 2; the subtraction pulse is equivalent to 2 picture elements and no particle is counted whose size is less than this value. Therefore three particles are counted.

(*c*) Sizing unit set on $2\sqrt{2}$; no particle is counted whose size is less than 2·83 picture elements; therefore two particles are counted

(*d*) Sizing unit set on 4; only one particle is counted.

(*e*) Sizing unit set on $4\sqrt{2}$; no particles are counted.

The automatic sizing is performed by the sizing unit, which produces a pulse of selectable width that corresponds to a given size of particle on the sample. Any particle whose size is less than that selected will not be recorded during a field count, so that by successive counts with selected pulses of progressively greater width a complete size analysis of the sample in the field can be obtained. The standard pulse widths obtained from the sizing unit are given in terms of picture element size, and increase in a $\sqrt{2}$ progression; the picture element size, of course, varies with the magnification used in the optical system. The operation of the sizing units is illustrated diagrammatically in Fig. 4.

When counting, one scan across the field takes 8 seconds, and a further complete scan has to take place to re-set the sizing unit. The total time taken to count each size range is, therefore, 16 seconds, and a complete size analysis of the field may take up to 3 minutes (if all the size ranges have to be explored). Normally, between 10 and 25 fields must be sized to obtain a statistically sound size analysis of the whole sample, the number of fields depending on the particle density of the sample and on its degree of heterogeneity. Preparation of the instrument for a particular type of sample may take from 15 to 30 minutes; many similar samples can be measured with little further adjustment of the instrument. The total time taken to count and size a sample is usually between 10 and 60 minutes.

ACCURACY AND RELIABILITY OF THE METHOD—

The accuracy of the instrument in counting and sizing any particular sample will depend on two main possible sources of error. First, errors within the instrument itself, *i.e.*, those inherent in its design or those due to faulty operation. Measurement on the reliability and accuracy of the instrument showed it to be somewhat better than visual measurement on similar samples, and operating errors, with reasonable experience, were negligible.[4]

The second and by far the more important source of error in automatic counting and sizing lies in the nature of the sample. It has already been shown that aggregates and irregularly shaped particles can produce spurious counts, and it must be remembered that any form of artificial scanning can never be as discriminating as the human eye. An ideal sample for automtic counting with this instrument should have the properties listed below.

1. A reasonable degree of contrast between particle and background; a minimum of 10 per cent. optical contrast and preferably more.

2. The particles should have clear-cut, well defined edges; any haziness or reduction in contrast towards the edges will affect the length of the pulse that the particle produces, and this cannot always be overcome by adjustment of the instrument.

3. The optical contrast should be reasonably uniform over each individual particle, *e.g.*, emulsion globules that appear under transmitted light as black rings with light centres will be counted as several particles.

4. The particles should be regular in shape and preferably circular (or spherical). Ellipsoidal or irregular shapes can be counted and sized to produce a mean or average figure provided the particles are randomly distributed on the sample. Very irregularly shaped particles may be counted more than once; the counts are registered by a bright spot appearing on the monitor screen so that these false counts can be seen and may be corrected visually.

5. The sample must not be overcrowded, because the scan will not differentiate between the individual particles in groups and aggregates. On the other hand, the samples should not be too sparse, or the total count per field would be low and many fields would have to be counted and much time wasted. The errors due to overcrowding may not be very important when a number frequency is to be measured, but when volume or mass parameters are to be calculated (and this is frequently the parameter of greatest interest in the pesticide field) the errors can be extremely serious.

To summarise, the speed, reliability and degree of accuracy of the automatic instrument are superior to those of visual counting. The instrument is versatile in both the range of particulate matter it can measure and in the range of magnification that can be used. Particles from 1 μ upwards may be measured by transmitted light and from 10 μ upwards by reflected light. These ranges may be extended by using an intermediate photographic process.

Thus the use of such an instrument removes most of the difficulties involved in the measurement of particle size, but it tends to increase the difficulties involved in obtaining a sample of suitable quality. It is the quality of the sample that is important; normal sampling errors can be reduced because the increase in speed of counting permits more particles to be measured than is usually possible with visual counting.

SAMPLING—

The sampling of sprays and dusts can be carried out in the laboratory by allowing them to settle out in a sedimentation chamber on to suitable collecting surfaces. The density of particles on the sample can be readily controlled to avoid undue overcrowding. Dust particles are relatively easy because they can be collected and measured directly on glass slides. Spray droplet sampling is more complicated in that the droplets will tend to spread when they impact on a collecting surface, and the droplets themselves must form a coloured stain that provides sufficient contrast to distinguish them from the background. In agricultural sprays, the latter requirement is best covered by adding a dye to the spray liquid; the degree of spread is dependent to a large degree on the nature of the sampling surface. Collection on various types of paper surfaces, commonly used in droplet sampling, is unsatisfactory for automatic counting, because the edges of the stains are always diffuse and the spread factor is often extremely large. Glazed photographic paper gives stains that are ideal for automatic counting, but there the stain size varies with the impaction velocity of the drop as well as with its size. Up to the present, siliconed surfaces, as described by Courshee,[7] have proved to be the most satisfactory in terms of quality of sample and constancy of spread factor.

Under practical spraying conditions in the open, sampling is very much more difficult and it is almost impossible to get a perfect size spectrum of a spray or a dust cloud. Besides the difficulties of sampling under isokinetic conditions, the size of a practical spray or dust cloud makes it virtually impossible to get samples representative of the whole cloud.

The automatic scanning technique is particularly valuable in determining the degree of cover obtained on sprayed or dusted surfaces. This is not necessarily concerned with the measurement of particles or microscopic stains because, with sprays particularly, the droplets may run together to give a more continuous film of deposit. Deposit areas can be measured by ignoring the counting and sizing circuits and electronically timing the total period during one scan that the scanning spot is obscured by the deposit. The time necessary for one complete scan with a completely clear field can also be measured, and the ratio of these two times gives the proportional area covered by the deposit. The samples for such measurements may be collected on artificial surfaces, but the most valuable information on coverage can only be obtained by studying the deposit on the surface that is used in practice, *i.e.*, the leaf. The measurement of samples collected on leaf surfaces presents serious difficulties, but these can be overcome by incorporating a fluorescent material into the spray or dust and then photographing the sprayed or dusted leaves in ultraviolet light.[8] The areas of deposit show up black on a light background, as shown in Fig. 5, and the photographic negative can be directly scanned to measure deposit area. Considerable practical difficulties arise in selecting the most appropriate fluorescent tracer to use in a particular pesticide formulation and the way to incorporate it into the formulation. It is essential that it will trace the pesticide deposit closely, because the area covered by pesticide deposited from sprays may be less than the total area wetted by the aqueous phase of the spray.

The measurement of oil droplets and solid particles dispersed in water poses some rather different problems. The automatic scanning of such particles is reasonably straightforward provided that the particles all lie in the same plane and, for emulsions, that the dispersed phase is suitably coloured. The first of these requirements can be met by placing a sample of the emulsion or suspension in a suitable cell, *e.g.*, a haemacytometer slide, and allowing the particles to rise to the surface or settle to the bottom of the cell before measurement. If the particles are extremely small and subject to Brownian motion, the sample can be photographed and the measurements made from the negative.

One of the greatest difficulties in this sampling process lies in extracting the sample from the bulk emulsion or suspension in a way such that it completely reproduces the particle size spectrum at the point of sampling. The samples are usually required at successive time intervals from the moment of preparation and at various depths below the surface of the emulsion or suspension, but the act of removing a sample can cause a considerable disturbance. Also aggregates or large emulsion globules in the extracted sample may be broken down by rapid passage through tubes or by passage through a narrow aperture.

This brief review of the most usual types of particulate matter encountered in pesticide formulation has dealt essentially with aqueous dispersions. However, it is also necessary to consider sprays of oil solutions, suspensions of solid particles in oil, and even multiple dispersions of suspensions of solid particles in oil that is itself emulsified in water. The measurement of particle size in any of these systems remains the same in principle, but different sampling problems may be involved. The measurement of particle size by automatic methods is fairly straightforward, but it is the obtaining of good samples that raises the greatest difficulties.

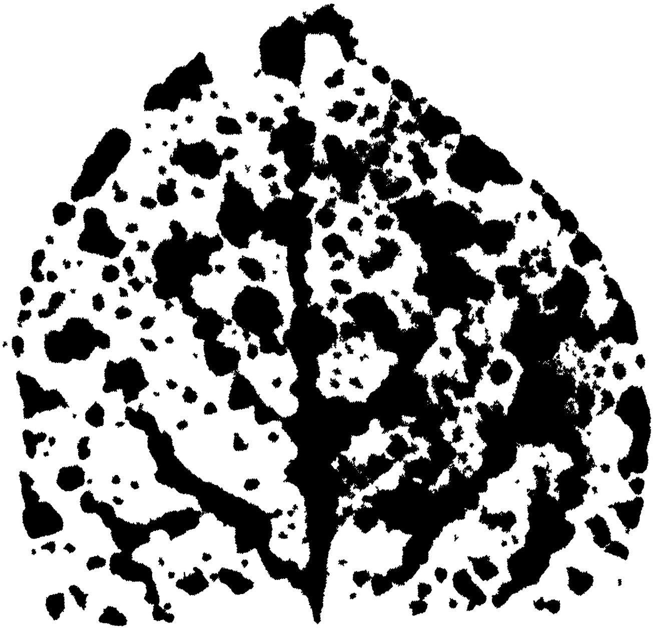

Fig. 5. Photograph of a fluorescent spray deposit on a leaf surface under ultraviolet light

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The Continuous Automatic Microbiological Assay of Antibiotics

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The principle of continuous culture of the test organism is applied to the AutoAnalyzer method for the microbiological assay of antibiotics by measurement of respiratory carbon dioxide. The use of *E. coli* as a general purpose test organism is described, and the method is extended to the assay of high potency samples of benzylpenicillin, streptomycins, neomycin, novobiocin and cephalosporin C. A modification is suggested for samples of lower potency. In comparison with a conventional agar-diffusion method (8 × 8 quasi-latin square), the AutoAnalyzer results showed no bias and, with duplicate recording of each sample, were of greater precision.

An AutoAnalyzer instrumental system for the microbiological assay of tetracyclines and polyene antibiotics has been described by Haney *et al.*[1] and Gerke, Haney and Pagano.[2] The method is based on measurement of the carbon dioxide resulting from free respiration by the test organism during a fixed incubation time and of the depression of respiration by graded concentrations of the antibiotic to be determined. In preliminary trials with the method it became clear that the instrumental system was satisfactory, but we encountered considerable difficulties in preparing a standard inoculum of a test organism (*Escherichia coli*) similar to that described for the assay of tetracyclines. It has been specified[1,2] that the organism be grown by overnight culture in a vigorously shaken medium; the cells are then collected by centrifugation, washed and re-suspended in fresh cold nutrient medium. Alternatively, the culture may be suitably diluted without centrifugation.[3] The inoculum produced in this way is maintained at 0° to 3° C during the working day in order to reduce to a minimum respiration of the cells in the inoculum reservoir.

The number of cells produced by overnight culture and their vigour depend on the degree of aeration the medium receives, and any variation in aeration necessitates a corresponding variation in the concentration of cells required for the working inoculum to maintain constant production of carbon dioxide in the AutoAnalyzer system. Moreover, the degree of sensitivity towards a given antiobiotic appeared to vary with the numbers of cells obtained, and this made it necessary to re-check for each inoculum the concentration of antibiotic required to give the working mid-point of the dose - response curve. Considerable time was often required at the beginning of the working day to establish the inoculum dilution and antibiotic concentration required for both standards and samples.

To assay certain antibiotics to which *E. coli* grown in this way is not sensitive we wished to use *Bacillus subtilis*. When grown overnight, cultures of *B. subtilis* contain a large proportion of spores, and a suitably diluted inoculum showed a rapid decline in ability to produce carbon dioxide when maintained at 0° to 3° C. Much younger cultures (4 hour), predominantly in the vegetative form, showed a similar rapid decline in vigour.

These difficulties led us to consider the possibility of continuous culture of the test organism; if successful, this would provide a vigorous and consistent inoculum for the assay and eliminate the need for maintaining a supply of ice-cold inoculum. The size of the reservoir required for continuous growth of the inoculum depends on the generation time of the organism at the incubation temperature and on the throughput of nutrient medium. With the short generation time of *E. coli* (about 20 minutes), a reservoir volume of 50 ml and the continuous addition of 0·8 ml per minute of sterile medium permits a suitable concentration of organisms to be maintained, and the whole unit can be accommodated in the AutoAnalyzer incubation bath. Vigorous aeration serves to mix this volume of culture and to remove both the respiratory carbon dioxide as it is formed and culture in excess of that required for the assay. The sensitivity of the organism grown in this way towards certain antibiotics, notably cephalosporin C and, to a lesser extent, benzylpenicillin, differs markedly from that of the same organism grown in overnight cultures.

METHOD

APPARATUS—

AutoAnalyzer—This consists of a modified sampling unit, a 15-channel proportionating pump, an incubation bath at 37° C, a colorimeter fitted with a 15-mm tubular flow-cell and 550-mμ filters and a single-pen recorder.

Fit the sampler with a reverse sampling crook and with a modified cam lever to provide a 2½-minute sample aspiration time. The constant-level device (see Fig. 2) should be clamped to the sampler, so that formaldehyde is aspirated for 24 seconds in each three-minute cycle.

Fix to the lower side of the cover of the bath four support pillars, approximately 7 inches in length, through the four central holes provided in the cover, and attach the lower end of the pillars to a 4-inch diameter disc of non-corrodible metal, suitably drilled. To one inlet of the cover attach 100 feet of polythene tubing, 0·110 inch internal diameter (Portex tubing 54B, Portland Plastics Ltd., Hythe, Kent), coiling it round the supports. Attach a glass T-joint to the other end of the tubing. To another inlet in the cover attach a short length of polythene tubing, a ½-length mixing coil and another length of polythene tubing to connect with the lower limb of the T-joint. Connect the remaining limb of the T-joint to two mixing coils in series and then to one of the outlets in the cover. For making polythene-to-glass connections, the ends of the tubing may be softened by brief immersion in boiling water, the tubing being then pushed over the glass fitting and secured with thin copper wire.

Fit the prepared inoculum reservoir (see below) through the hole in the rear of the incubation bath-top, and clamp in position.

Magnetic diaphragm air-pump—Obtainable from Chas. Austen Pumps Ltd., Byfleet, Surrey.

Inoculum reservoir and fittings—See Fig. 1.

Reagent reservoirs—Pyrex bottles, of 5-litre capacity, fitted with stoppers, each provided with one glass tube reaching to the bottom and an inlet connected to a gas wash-bottle containing sodium hydroxide solution.

REAGENTS—

Prepare all reagents with carbon dioxide free distilled water. Reagents 1, 3 and 4 are conveniently stored in 5-litre containers protected from atmospheric carbon dioxide.

1. *Sulphuric acid*, N—Add 0·1 per cent. v/v of MS Silicone Antifoam Emulsion RD (Hopkin & Williams Ltd., Chadwell Heath, Essex) to N sulphuric acid.

2. *Carbonate buffer*—Dissolve 56 g of analytical-reagent grade sodium hydrogen carbonate and 35 g of analytical-reagent grade anhydrous sodium carbonate in sufficient water to produce 1 litre.

3. *Buffered phenolphthalein reagent*—Add 7·0 ml of a 1 per cent. w/v solution of phenolphthalein in methanol to about 900 ml of water, and mix. For assay with *E. coli* 397E as test organism, add 5·5 ml of carbonate buffer, and dilute with water to 1 litre. Add 0·25 ml of capryl alcohol, and shake well. Adjust the strength of this reagent as described on p. 698.

4. *Diluent*—Add 0·1 per cent. v/v of Tween 20 to distilled water, and mix.

5. *Formaldehyde solutions*, (a) 1 *per cent. w/v*, (b) 0·1 *per cent. w/v.*

(*a*) Dilute 2·5 ml of a 40 per cent. w/v solution of formaldehyde to 100 ml with diluent.

(*b*) Dilute solution (*a*) (1 + 9) with diluent.

NUTRIENT MEDIA—

Medium 1—Prepare from the materials listed below.

Dehydrated Penassay broth (Difco*)	17·5 g
Tryptone (Difco*)	10·0 g
Yeast extract (Difco*)	5·0 g
Polypropylene glycol, P2000	0·02 ml
Distilled water	to 1 litre

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* Equivalent materials can be substituted, but they may not necessarily give the same yield of carbon dioxide, the same sensitivity to the antibiotic or the same dose - response slope.

Sterilise 1·5-litre amounts in 80-oz bottles in an autoclave at 121° C (15 lb. per sq. inch) for 20 minutes. The bottles should be covered with aluminium foil during the sterilisation and then sealed immediately with sterile rubber caps. Store, preferably at 4° C.

Medium 2—Prepare exactly as described for medium 1 except for the substitution of 2 ml of Tween 20 per litre in place of the polypropylene glycol. Sterlilise 2-litre amounts in 80-oz bottles. Store, preferably at 4° C.

TEST ORGANISMS—

As a general-purpose test organism, suitable for assaying many antibiotics, *E. coli* 397E has proved satisfactory, but the principle of continuous culture is of general application to other organisms including spore-formers. For such organisms the volume of the reservoir and the input of medium may be adjusted so that the inoculum used in the assay consists almost entirely of organisms in the vegetative form. Whatever organism is selected it must be capable of rapid growth and respiration and must be of sufficient sensitivity to the antibiotic being assayed. The sensitivity should be assessed under continuous culture conditions, since other methods for measuring the sensitivity to an antibiotic are not necessarily relevant.

Maintain test organisms as agar-slope cultures, fresh 24-hour slope cultures being prepared as required for the assay.

PREPARATION AND CONTINUOUS CULTURE OF INOCULUM—

Attach non-absorbent cotton filters to the air-inlet tube of the inoculum reservoir (see Fig. 1) and to the inlet side of the fittings for the reservoir of medium 1. Wrap the fittings, G, of the reservoir assembly in aluminium foil, and cover the tops of tubes C and E with foil. Sterilise the whole assembly in an autoclave at 121° C (15 lb. per sq. inch) for 20 minutes.

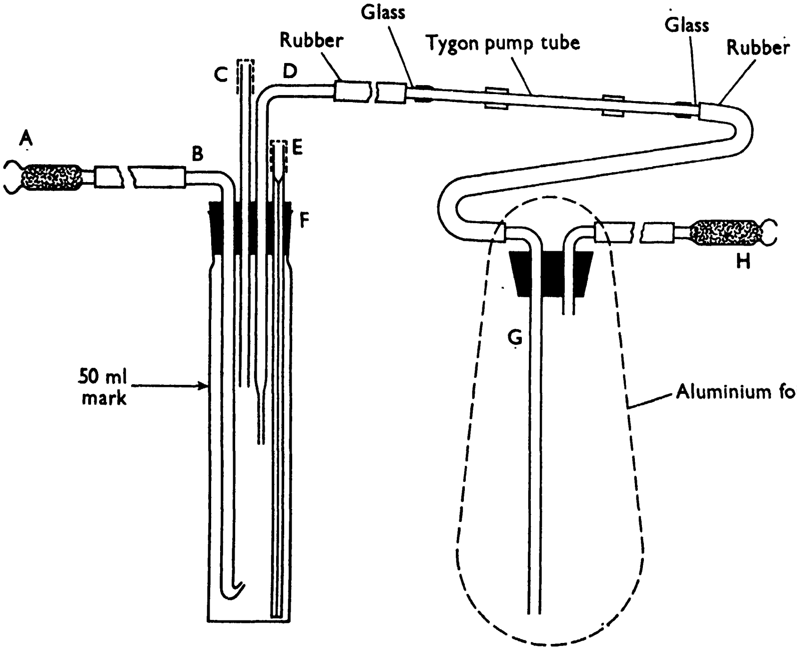

Fig. 1. Assembly for continuous culture of inoculum

When required for use, aseptically remove the foil film from assembly G, and insert into a bottle of sterile medium 1. Slightly ease out the plug on the inoculum reservoir, and by means of air-pressure applied to the filter, H, blow over 50 ml of medium 1 into the inoculum reservoir.

Aseptically remove 5 ml of medium from the inoculum reservoir, and add it to an overnight agar-slope culture of the test organism. Suspend the organisms in the medium, and transfer the suspension to the inoculum reservoir. Re-secure the plug and fittings, ensuring that the jet of the air-inlet tube B is directed round the circumference of the reservoir and away from the bottom of the inoculum tube E. Immerse the inoculum reservoir in the incubation bath, and clamp in position, so that the level of liquid in the reservoir is below the level of the water in the bath. Insert the Tygon pump tube into one channel of the AutoAnalyzer pump, and start the pump. Connect the air-pump to filter A, and pass a steady stream of air (about 40 litres per hour) into the inoculum reservoir. Allow the system, assembled as described below, to reach equilibrium, as will be shown by the production of a constant amount of respiratory carbon dioxide during the fixed incubation time of the assay. For organisms with a short generation time, 6 hours should be sufficient, but it is convenient to allow equilibration to proceed overnight.

The inoculum can be renewed at the end of each working day or allowed to continue for several days by aseptically transferring the assembly G to a fresh bottle of medium 1 each day. Alternatively, a reservoir of medium 1 large enough to last for a week may be attached, provided contamination of the medium and inoculum reservoir can be prevented.

ASSEMBLY OF AUTOANALYZER—

The assembly shown in Fig. 2 incorporates a preliminary 1 + 9 dilution stage. This is optional but convenient for the assay of solid samples with potencies approaching those of the pure antibiotics, since the need for making many accurate dilutions by hand is thereby avoided. If, however, greater sensitivity is desired, the preliminary dilution stage can be omitted, as shown in Fig. 3, or a smaller dilution can be substituted Whatever arrangement is adopted, it is essential that the variable amount of air admitted by the alternating operation of the antibiotic and formaldehyde sample tubes be eliminated from the system. Only liquid may be permitted to enter the re-sampling tube.

If the preliminary dilution does not exceed about 1 + 9 the whole manifold can be pumped satisfactorily with one pump, but it may be more convenient to use a separate pump for the medium 1 and the preliminary dilution stage, particularly if a larger preliminary dilution is desired.

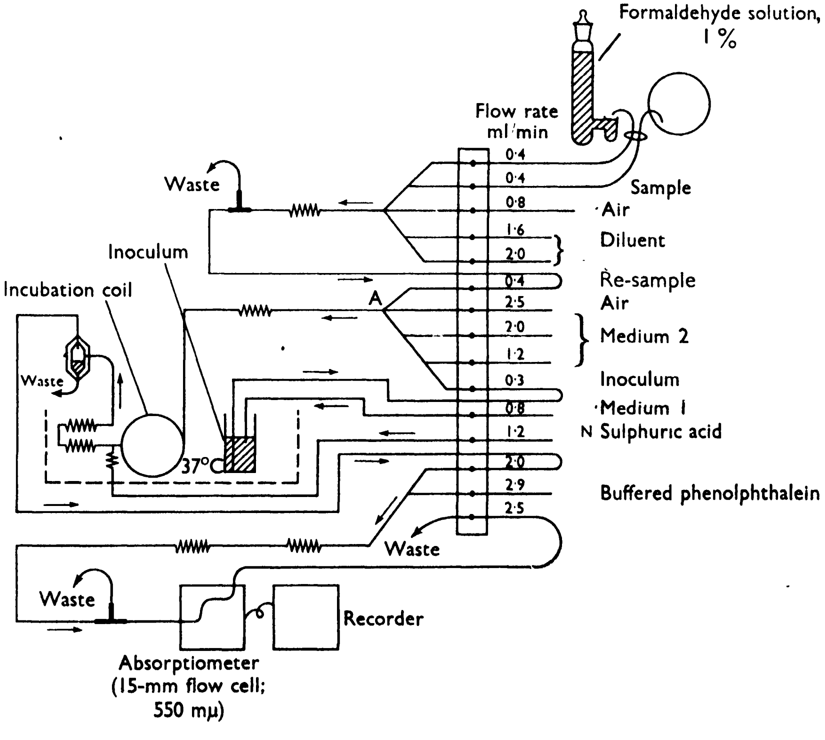

Fig. 2. Flow diagram for automatic microbiological assay of high-potency antibiotic samples

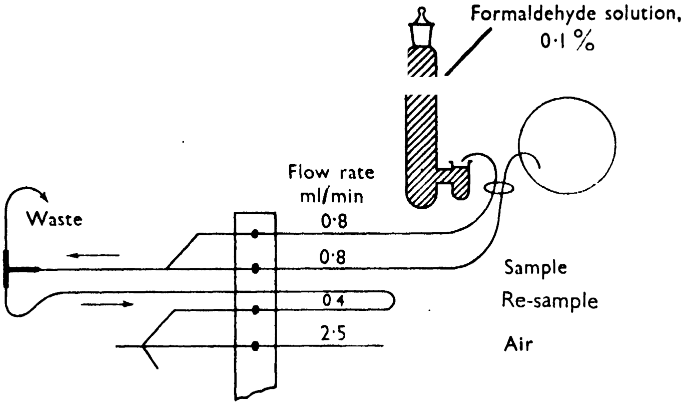

Fig. 3. Partial flow diagram for increased sensitivity

Apart from the continuous culture assembly the rest of the AutoAnalyzer arrangement is substantially that suggested by Haney, Gerke, Madigan, Pangano and Ferrari.[1] However, we prefer to carry out the gas - liquid separation at 37° C with the vacuum-jacketed separator shown in Fig. 4; this, if mounted immediately on top of the incubation bath, permits visual examination of its performance. The substitution of a 15-mm tubular flow-cell in place of the 10-mm conventional one gives improved separation of samples and a better dose - response calibration curve.

The glass cactus-fitting, A (see Fig. 1), must be of sufficiently wide bore to generate air bubbles large enough to maintain a regular bubble pattern throughout the incubation coil.

To set up the AutoAnalyzer for the assay, fill the constant-level device with 1 per cent. formaldehyde solution (or 0·1 per cent. formaldehyde solution if the preliminary dilution is omitted, as shown in Fig. 3), and connect tube E of the inoculum reservoir to the inoculum tube of the manifold with 0·045-inch bore polythene tubing. This connection should be as short as possible, to minimise the continued growth of the organisms before joining the medium 2 and diluted sample streams.

Connect the appropriate tubes to the diluent, N sulphuric acid, medium 2 and reagent reservoirs, making the connection to the buffered phenolphthalein reagent with 0·045-inch bore polythene tubing. Set the sample-plate in operation at 20 tests per hour, with water in the sample-cups. When peaks begin to be recorded, adjust the strength of the buffered phenolphthalein reagent with either carbonate buffer or distilled water containing 0·7 per cent.

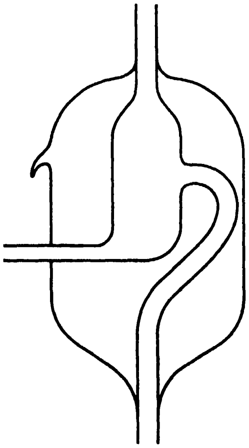

Fig. 4. Vacuum-jacketted gas - liquid separator, working capacity, approximately 0·7 ml

v/v of a 1 per cent. w/v solution of phenolphthalein in methanol, so as to obtain peak transmissions of 90 per cent.

Determine the concentration of the antibiotic under test required to give the mid-point of the dose - response curve (50 per cent. transmission) by placing a series of suitable dilutions in the sample-cups, each dilution being placed in three successive cups. Approximate mid-point concentrations for some antibiotics (with the preliminary 1 + 9 dilution) can be read from Fig. 5. Re-establish the mid-point concentration (medium standard) weekly or when any change is made in the manifold. Once this is established the concentration of the buffered phenolphthalein reagent may be adjusted to give 50 per cent. transmission with the medium standard in the sample cups.

Haney *et al.*[1] recommended that the concentration of the high and low standards, required to establish the slope of the dose -response curve, be $1\frac{1}{2}$ times and $\frac{2}{3}$ of the concentration of the medium standards, respectively. For the arrangement shown in Fig. 2 and with continuous inoculum culture, a narrower range of concentration is necessary to retain the responses on the approximately linear portion of the dose - response curves (preferably within the limits of 35 to 65 per cent. transmission).

The permissible ratio depends on the slope; for streptomycin, suitable concentrations are $\frac{6}{5}$ and $\frac{5}{6}$ of that of the medium standard, giving, for example, cup concentrations of 3·0, 2·5 and 2·08 mg per ml for the three standards.

At the end of each working day disconnect the inoculum tube from the inoculum reservoir, and plug the top of tube E. Pass 2 N sodium hydroxide through the inoculum, medium 2 and buffered phenolphthalein tubes for 5 minutes, and then pump diluent through to clear any deposit from the incubation and mixing coils.

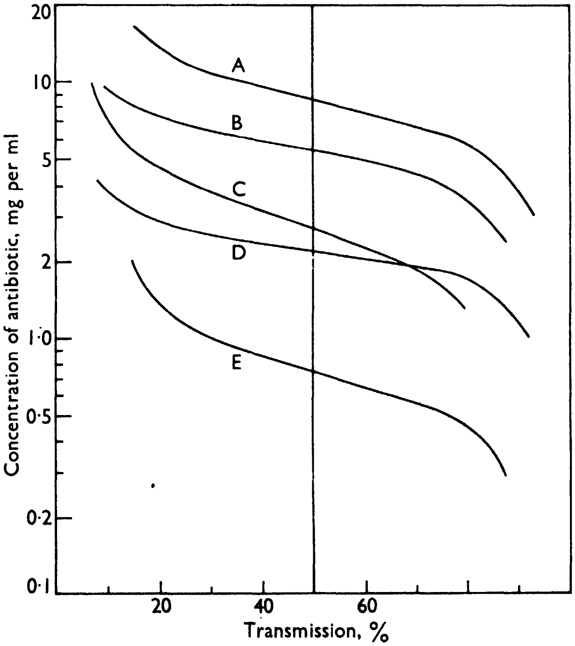

Fig. 5. Calibration graphs : curve A, novobiocin; curve B, sodium benzylpenicillin; curve C, streptomycin sulphate; curve D, sodium cephalosporin C; curve E, neomycin sulphate

ASSAY DESIGN—

In the design proposed by Haney *et al.*[1] each sample-plate is loaded with a series of 4 high, 4 low and 4 medium standards, 5 samples and 2 medium standards, with the last two (5 samples and 2 medium standards) being repeated three more times. Each sample is assayed in duplicate. The responses of the medium standards are connected on the recording by a line (the drift line), and the responses of all samples are measured as differences from this line. The slopes of the dose - response curve above and below the medium standard are determined, and these permit calculation of the potencies of samples as differences from that of the medium standard.

Many different assay designs are possible according to the replication, and hence the precision, desired in relation to throughput of samples. In one design, which permits mathematical correction for drift, the slope of the dose - response curve over the narrower range of potencies suitable for the AutoAnalyzer arrangement described above is first established with 4 low and 4 high standards. The remaining sample-plate positions are then filled with two similar 16-position patterns, each of 6 samples in duplicate and 4 medium standards. The samples and standards are so arranged that the second eight solutions in each pattern are in the reverse order of the first eight. Thus solutions in the order A to H are immediately followed by the same solutions arranged H to A. The mean response for each sample and for the four medium standards is then independent of drift, if it is assumed that this is linear over the 48 minutes required for recording the 16 responses. The medium standards may occupy any positions in the pattern, but are conveniently placed in positions B and F.

Both the designs considered above are one-level (1 × 1) assays, in which the slopes of the dose - response lines for the standard and for every sample are known to be the same or when this may be assumed. Moreover, with *E. coli* 397E as test organism similar slopes are given by different antibiotics (Fig. 5), and it is therefore necessary that the qualitative composition of the sample be known.

CALCULATION OF RESULTS—

Read off from the recording the percentage transmission (T%) for the low and high standards, ignoring for each the first of the four responses. Calculate the mean response for each standard, determine the difference, D, and calculate the slope constant, K, from the equation $K = \frac{R}{D}$, where R is the log of the dose ratio (high to low). The slope constant represents on a log scale the concentration difference for each 1 per cent. transmission over the working range and is positive or negative according to whether the response of a sample (in T%) is numerically less or more than that of the medium standard.

Calculate the mean responses for each sample and for the four medium standards. Determine the difference in T% from the medium standard for each sample, and multiply

TABLE I

STREPTOMYCIN ASSAYS

Sample number	Prepared strength of streptomycin sulphate, mg per ml	Amount of streptomycin found by using—				
		AutoAnalyzer			Agar-plate method	
		1,* mg per ml	2,* mg per ml	3,† mg per ml	1,‡ mg per ml	2,‡ mg per ml
1	3·00	3·01	3·00	2·95	2·85	3·05
2	3·50	3·46	3·52	3·42	3·47	3·46
3	3·20	3·17	3·20	3·08	3·30	3·28
4	3·10	3·08	3·09	2·99	3·07	3·03
5	2·90	2·83	2·93	2·79	2·99	2·88
6	2·60	2·60	2·54	2·61	2·54	2·43
7	2·70	—	2·64	—	2·76	2·87
8	3·16	—	3·17	—	3·11	3·41
9	3·20	—	3·34	—	3·30	3·27
10	3·52	—	3·60	—	3·51	3·51
11	2·86	—	2·90	—	3·03	2·85
12	2·78	—	2·77	—	2·93	2·78

* Calculated by the proposed method.

† Calculated by the method of Haney *et al.*[1]

‡ The results of an 8 × 8 quasi-latin square design with *B. subtilis* 841 as test organism.

by K, maintaining the correct sign. Add this to (or subtract it from, as appropriate) the log of the potency of the medium standard. Convert to the antilog to give the concentration of the sample solution, and calculate the result on the original sample.

The results shown in Table I were obtained on a series of accurately prepared dilutions (unknown to the operator) of streptomycin sulphate by the two AutoAnalyzer designs and methods of calculation discussed above. In comparison with the known concentration and with results on the same samples assayed at the same time by a conventional two-level agar-diffusion method (8 × 8 quasi-latin square) with a different test organism, the AutoAnalyzer results show no bias and demonstrate that, with the duplicate recording of samples recommended, an appreciably lower error can be expected.

We thank all those at the Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A., who generously supplied advance information of their procedures, Mr. W. K. Anslow, Dr. A. Ferrari and Mr. J. P. R. Tootill for helpful discussions and Mr. K. Clover for assistance with the practical work.

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Automatic Procedures for the Colorimetric Analysis of Iron- and Steel-making Slags

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The AutoAnalyzer system of automatic colorimetric analysis has been successfully applied to the determination of total iron and the oxides of manganese, phosphorus and aluminium in iron- and steel-making slags. The sample is decomposed in acid, silicon removed as metasilicic acid and the solution diluted to a fixed volume. From this stage analysis is completely automatic, the operator being required only to read optical-density values from a chart recorder and convert these into percentage content by reference to a calibration factor. The automated procedures are primarily intended for analysing at least ten sample solutions at a time. Each slag constituent is determined separately by using a specially designed flow system that can be assembled and calibrated in about 40 minutes. The speed of operation is either 20 or 40 samples per hour dependent on the type of chemical reaction involved.

THE use of the Technicon AutoAnalyzer is well established in clinical and industrial analysis. Typical applications of this equipment in the United Kingdom have included the determination of streptomycin in fermentation broths[1] and the determination of zinc, lead, molybdenum and nickel in soil extracts.[2]

The sample in solution form is aspirated, pumped through the apparatus and mixed with appropriate reagents to produce a coloured solution. This coloured solution is passed through a colorimeter; the optical density is recorded on a chart and related to percentage content from a calibration graph. A fuller description of the mechanics and principles of operation has been provided by Ferrari, Russo-Alesi and Kelly.[3]

As an initial study of the instrument's application to steelworks' materials, four spectrophotometric methods for analysing iron- and steel-making slags have been automated. These methods form part of an analytical scheme used in our laboratory,[4] in which the sample of slag is decomposed in acid, silica removed and the solution diluted to a fixed volume. From this solution, separate portions are taken for the determination of total iron, manganese oxide, phosphorus pentoxide and alumina by spectrophotometry, and calcium and magnesium oxides by complexometric titration. This paper described the adaptation of the spectrophotometric procedures for use with the Technicon AutoAnalyzer.

PREPARATION OF SAMPLE SOLUTION

A powdered slag sample weighing 0·5 g is partly decomposed in hydrochloric acid; the solution is then oxidised, perchloric acid is added, and the solution is evaporated until fumes of perchloric acid are evolved. Fuming is continued under reflux for 10 to 15 minutes to separate silicon as metasilicic acid. After removal by filtration, the precipitate is ignited and weighed as silica. Finally, the silica is volatilised as the fluoride, the residual oxides being fused and added to the sample solution. This solution is evaporated to fumes for a second time to remove hydrochloric acid and to oxidise chromium to the sexavalent state. The volume of perchloric acid remaining should be about 10 to 15 ml, giving an approximately 2·5 per cent. v/v acid solution on dilution to 500 ml. Full details of the preparation of the sample solution are given in a British Iron and Steel Research Association Publication.[4]

DETERMINATION OF ALUMINA

In the manual method, iron and manganese are separated from aluminium by precipitation with sodium hydroxide solution in the presence of hydrogen peroxide. Interference due to titanium and vanadium is prevented by a further addition of hydrogen peroxide. After the solution has been carefully neutralised and adjusted to pH 6·0, Eriochrome cyanine is added to produce the orange-red aluminium - dye complex; colour development is complete in 20 to 30 minutes.

Initial tests indicated that the colour reaction could be automated, provided that two time-delay coils were placed in the flow system to allow sufficient time for reproducible colour development. The colour reaction is extremely sensitive, and it was difficult to design a flow system to cover the desired percentage range (up to 15 per cent. of alumina, equivalent to 8 mg of aluminium per 100 ml of sample solution). Considerable dilution was necessary, and this was achieved by a method of "opposed dilution" introduced by Technicon Instruments Ltd. In the proposed system, a tube pumping diluent at 0·32 ml per minute in opposition to a sample line pumping at 0·42 ml per minute gives an effective sample intake of 0·1 ml per minute. (A tube of flow rate 0·32 ml per minute was the smallest available to us when these procedures were developed.) With a high over-all flow rate of 18 ml per minute, it was now possible to cover at least half the desired percentage range. For concentrations greater than about 7·5 per cent. of alumina, a diluted sample solution must be used.

Considerable difficulties were caused by excessively noisy records. This was traced to the formation of a blue deposit in the flow-through cell of the colorimeter and was probably due to the decomposition of the aluminium - dye complex. This difficulty was overcome by adding a small amount of acetone to the dye solution.

Contamination between successive samples is not a serious problem in the AutoAnalyzer system provided that a properly designed manifold of tubes is used. The problem is, however, accentuated when such strongly coloured dye solutions as Eriochrome cyanine are used. For this reason, it is preferable to use alternate plastic cups filled with dilute perchloric acid to provide a thorough washing after each sample has been analysed. An additional advantage of this technique is the improvement in resolution between successive peaks on the chart recorder. In the proposed flow system, sampler-plate operating at 40 tests per hour with alternate acid-filled cups gives an effective speed of 20 samples per hour.

INTERFERING ELEMENTS—

In an attempt to automate the procedure for removing iron, etc., in the manual method, the sample stream was mixed with dilute solutions of sodium hydroxide and hydrogen peroxide, and dialysed through a Cellophane membrane. Only partial success was attained since there was frequent rupturing of the membrane. In subsequent work, the use of ascorbic acid for complexing iron made it possible to dispense with the dialyser unit.

Ascorbic acid was first proposed by Hill[5] as a suitable complexing agent for iron and certain other elements in the aluminium - Eriochrome cyanine reaction. In our experience, the presence of this reagent during colour development in the manual procedure results in the formation of a coloured product that is not sufficiently stable with respect to time to permit optical-density measurements to be made. This latter consideration is of less importance in the AutoAnalyzer system, as all measurements are made under identical conditions, and, provided that the rate of colour development is reproducible, the production of

TABLE I

THE EFFECT OF VARIOUS ELEMENTS ON THE DETERMINATION OF ALUMINA

Interfering element	Amount of interfering element added, mg per 100 ml	Amount of aluminium found when— 0·2 mg of Al was added, mg per 100 ml	1·0 mg of Al was added, mg per 100 ml	3·0 mg of Al were added, mg per 100 ml
Titanium	1·0	0·25	1·00	—
	2·5	—	1·12	—
Manganese	20·0	0·25	0·98	—
	1·0	—	1·00	—
Chromium^{VI}	2·5	0·20	0·98	3·00
	10·0	—	0·89	—
	20·0	—	0·80	—
	1·0	—	1·02	—
Vanadium	2·5	0·18	1·02	—
	10·0	—	1·26	—
	20·0	—	1·49	—
Phosphorus	20·0	—	0·99	—
	50·0	0·20	1·02	—

a time-stable complex is not essential. Tests confirmed that in a flow system operating under closely controlled conditions (see Fig. 1) iron in amounts up to 25 mg per 100 ml of sample solution could be successfully complexed with ascorbic acid. There was, however, a slight positive interference, equivalent to about 0·05 mg of aluminium, when the amount of iron added was increased to 50 mg per 100 ml.

Previous experience in determining aluminium by using Eriochrome cyanine suggested that, of the other elements present in slags, titanium, vanadium, manganese, chromium and phosphorus might interfere in the colour reaction. The effect of these elements was determined at three concentration levels of aluminium, and the results are summarised in Table I. Interference was considered to be significant when the amount of aluminium found lay outside arbitrary limits of ± 0·03 mg at the 1·0 mg and 3·0 mg per 100 ml levels, and ± 0·05 mg at the 0·2 mg per 100 ml level when the analytical precision is not great owing to the sigmoid character of the calibration graph.

Titanium and vanadium in amounts of more than 1 mg and 2·5 mg per 100 ml, respectively, interfere seriously giving high results. It was not possible to mask interference of these elements with hydrogen peroxide as in the manual procedure, presumably because of the influence of the ascorbic acid present in the flow system. Chromium in the sexavalent state can be tolerated in amounts up to 2·5 mg per 100 ml, but with chromium contents above this level low results for aluminium will be obtained. Manganese up to 20 mg and phosphorus (as phosphate) up to 50 mg per 100 ml are without effect. In terms of the percentage oxide content, the limits are 2 per cent. of titania, 4 per cent. of chromium trioxide and 4 per cent. of vanadium pentoxide.

METHOD

REAGENTS—

Ascorbic acid, 0·5 per cent. w/v—Dissolve 5 g of ascorbic acid in water, add 10 drops of Teepol, and dilute to 1 litre.

Perchloric acid, diluted (1 + 3) *and* (1 + 39)—Prepare from perchloric acid, sp.gr. 1·54.

Dye solution—Dissolve 0·25 g of Merck Eriochrome cyanine in water, add 100 ml of acetone, and dilute to 1 litre in a calibrated flask. Prepare a fresh solution daily.

Buffer solution, pH 6·4—Dissolve 400 g of analytical-reagent grade hydrated sodium acetate in water, add 10 ml of glacial acetic acid, and dilute to 1 litre. The pH of this solution should be between 6·35 and 6·45. Store in a stoppered bottle.

Standard aluminium solution—Dissolve 0·5293 g of high purity aluminium metal in 20 ml of hydrochloric acid, add 15 ml of perchloric acid, and evaporate to fumes. Cool, add water, and warm, if necessary, to redissolve the salts. Dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 1 mg of alumina.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 1. Set the sampler-plate at the rate of 40 samples per hour, and fill alternate sample-cups with dilute perchloric acid (1 + 39). Prepare a series of calibration solutions by adding up to 10 ml of standard aluminium solution to 10 ml of diluted perchloric acid (1 + 3). Dilute each solution to 100 ml in a calibrated flask, and store in a tightly stoppered polythene bottle. Prepare a calibration curve by running the calibration solutions in duplicate. Draw the base line on the chart recorder by joining together the small peaks due to the intermediate acid cups, and record the peak height of each calibration solution in terms of optical density. The base line drifts slightly with time owing to fading of the dye solution, but the drift should not exceed 0·005 optical-density units per hour. Deduct the optical density of the reagent blank solution in each determination.

The graph relating aluminium content to optical density is sigmoid in character (see Fig. 2); it is, however, rectilinear in the range 1·5 to 7·5 mg of alumina per 100 ml, and the curve between these two points can be expressed as $y = mx + c$. For contents greater than 7·5 mg of alumina per 100 ml, the graph is curved and not reproducible. In routine use, a graph should be plotted with each batch of tests, but if the alumina content of the samples is known to exceed 2 per cent., *i.e.*, falling on the linear part of the graph, it is only necessary to run, say, three calibration solutions each day to establish its slope. At least one calibration solution should be measured with each sampler-plate of test solutions as a check on calibration drift.

Run the test samples through the apparatus, and calculate the percentage of alumina by reference to the calibration graph. Deduct the apparent percentage of alumina found in the reagent blank solution.

For samples that give optical-density values on the upper curved part of the graph, repeat the tests on a diluted sample solution containing sufficient added dilute perchloric acid to maintain the acid concentration at (1 + 39), *e.g.*, for a (1 + 1) dilution, take 50 ml of sample solution, add 5 ml of diluted perchloric (1 + 3), and dilute to 100 ml in a calibrated flask.

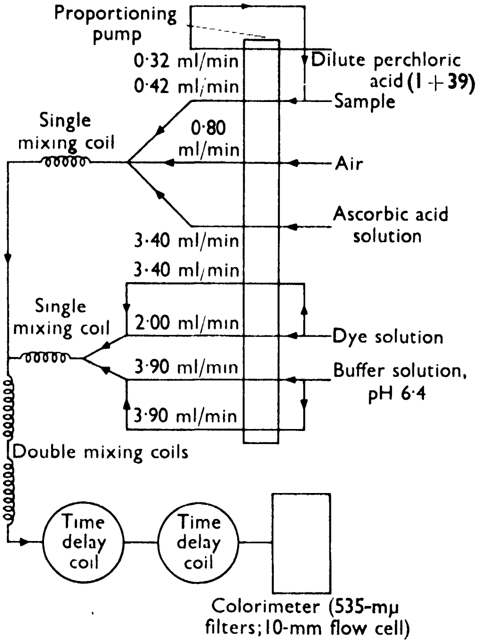

Fig. 1. AutoAnalyzer flow system for determining alumina in steel-making slags

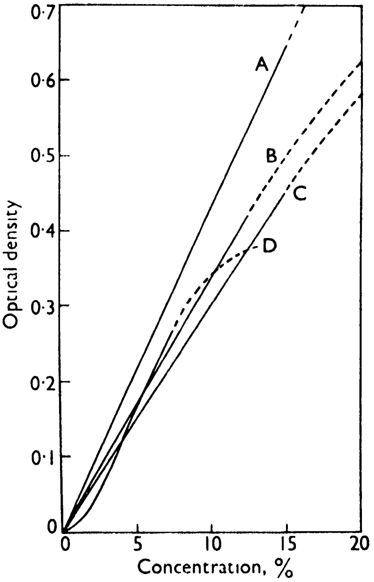

Fig. 2. Calibration graph for the analysis of steel-making slags with the AutoAnalyzer : curve A, total iron; curve B, manganese oxide; curve C, phosphorus pentoxide; curve D, alumina

DETERMINATION OF PHOSPHORUS PENTOXIDE

In the manual method, a solution containing ammonium vanadate, ammonium molybdate and dilute nitric acid is added to a portion of the sample solution to form the yellow molybdovanadophosphoric acid complex. This simple procedure proved easy to automate, but it was necessary to give some consideration to the choice of light filter and to possible interference from iron present in the sample solution. Initial tests indicated a slight positive interference from iron when the 440-mμ filter (corresponding to the wavelength used in the manual method) was used. At this wavelength the optical-density readings were rather low, *e.g.*, 0·21 optical-density units for solutions containing 5 mg of phosphorus per 100 ml, and for this reason the more sensitive 420-mμ filter was preferred (0·35 optical-density units for 5 mg of phosphorus per 100 ml). Unfortunately, interference from iron is more pronounced as the ultraviolet part of the spectrum is approached. A series of tests in which iron was added to solutions containing different amounts of phosphorus showed that interference was roughly proportional to the amount of iron present, up to phosphorus contents of 8 mg per 100 ml of test solution. Thus it is possible to make a small deduction from the apparent phosphorus content to allow for interference from iron; the deduction is 0·002 mg of phosphorus per mg of iron present.

METHOD

REAGENTS—

Molybdovanadate solution—Transfer 0·31 g of ammonium metavanadate to a beaker containing 50 ml of water and slowly add 40 ml of nitric acid, sp.gr. 1·42, while swirling the solution. Heat below the boiling-point until dissolution is complete. Dissolve 12·5 g of ammonium molybdate in 100 ml of water, warming if necessary. Cool both solutions thoroughly, mix, and dilute to 1 litre. Store in a stoppered polythene bottle; the solution is stable for about 3 days.

Standard phosphorus solution—Dissolve 1·917 g of potassium dihydrogen orthophosphate in water, dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 1 mg of phosphorus pentoxide.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 3, and set the sampler-plate at the rate of 40 samples per hour. Prepare a standard calibration graph from a series of solutions containing 10 ml of diluted perchloric acid (1 + 3) and up to 20 ml of standard phosphorus solution, diluted to 100 ml to cover a range of contents up to 20 per cent. of phosphorus pentoxide.

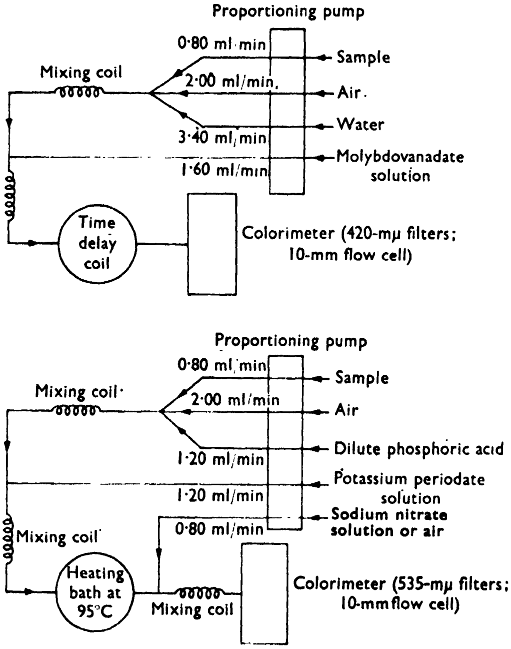

Fig. 3. AutoAnalyzer flow systems for determining (*a*) phosphorus pentoxide and (*b*) manganese oxide in steel-making slags

With the instrument used by us the calibration graph was linear up to 15 per cent. of phosphorus pentoxide (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

For routine use plot three calibration points in duplicate to establish the slope of the graph. At least one calibration solution should be measured with each sampler-plate of test solutions as a check on the calibration.

For sample solutions containing coloured ions, repeat the determinations substituting dilute nitric acid (4 + 96) for the molybdovanadate solution. Deduct these readings to give the net optical density due to phosphorus pentoxide.

DETERMINATION OF MANGANESE OXIDE

Manganese is determined manually by oxidising it to permanganic acid by heating with potassium periodate in a strongly acid solution. In the automated procedure, development of the manganese colour is effected by passing the solution through a 40 ft length of glass tubing in the form of a coil immersed in a heating bath maintained at 95° C. Provision is made for reducing the manganese colour with sodium nitrite in order to determine the optical density of any coloured ions, such as chromium, present in solution.

METHOD

REAGENTS—

Phosphoric acid, diluted (1 + 3).

Sodium nitrite solution, 0·5 *per cent. w/v, aqueous.*

Potassium periodate solution, 2 *per cent. w/v*—Transfer 20 g of potassium periodate to a 1-litre beaker, add 800 ml of diluted phosphoric acid (1 + 3), heat almost to the boiling-point, and stir until dissolved. Cool, and dilute to 1 litre with diluted phosphoric acid.

Standard manganese solution—Dissolve 1·1144 g of potassium permanganate in 500 ml of water, reduce with a slight excess of sulphurous acid, dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 0·5 mg of manganese oxide.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 3, and set the sampler-plate at the rate of 40 samples per hour. Prepare a standard calibration graph as described under "Determination of Phosphorus Pentoxide," by using suitable volumes of standard manganese solution to cover a range of up to 20 per cent. of manganese oxide.

With our instrument the calibration graph was linear up to 12·5 per cent. of manganese oxide (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

For a sample solution containing coloured ions repeat the determinations, substituting 0·5 per cent. w/v solution of sodium nitrite for the air-line immediately after the heating bath. Deduct these readings to give the net optical density due to manganese oxide.

DETERMINATION OF TOTAL IRON

In the manual procedure, iron is reduced to the bivalent state with hydroxyammonium chloride; the solution is buffered to about pH 4 with sodium citrate solution, and 1,10-phenanthroline hydrate is added to form the orange-red complex. Colour development is complete in about 20 minutes.

The two principal difficulties encountered in automating the manual method were contamination and lack of resolution between samples. The problem of contamination was overcome by introducing intermediate acid-filled cups between successive samples as in the method for alumina. In addition, an extra large-diameter air-line was included in the flow system to scavenge contaminants from the time-delay coil used for colour development. These modifications also considerably improved resolution between successive peaks on the chart recorder.

It was established that minor variations in the amount of perchloric acid in the main sample solution would not affect the optical density of the coloured solution provided that the acid content was between 2 per cent. v/v and 3 per cent. v/v; acid contents in excess of this range would lead to low results.

The slope of the calibration graph was found to depend on the concentration of 1,10-phenanthroline hydrate present in the system, but at all concentration levels the graph was linear up to 15 mg of iron per 100 ml of test solution. Above this value the graph is curved and non-reproducible (see Fig. 2).

METHOD

REAGENTS—

Hydroxyammonium chloride solution, 0·5 *per cent. w/v, aqueous.*

Sodium citrate solution, 3 *per cent. w/v, aqueous.*

1,10-*Phenanthroline hydrate solution*, 0·2 *per cent. w/v, aqueous.*

Standard iron solution—Dissolve exactly 1 g of Matthey iron sponge in 20 ml of hydrochloric acid, oxidise with nitric acid, add 10 ml of perchloric acid, and evaporate to fumes. Cool, add water, and warm, if necessary, to dissolve salts. Dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 1 mg of iron.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 4. Set the sampler-plate at 40 samples per hour, and fill alternate cups with dilute perchloric acid (1 + 39). Prepare a standard graph as described under "Determination of Phosphorus Pentoxide"; use suitable volumes of a standard iron solution to cover a range of contents up to 20 per cent. of total iron.

With our instrument, the calibration graph was linear up to 15 per cent. total iron (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

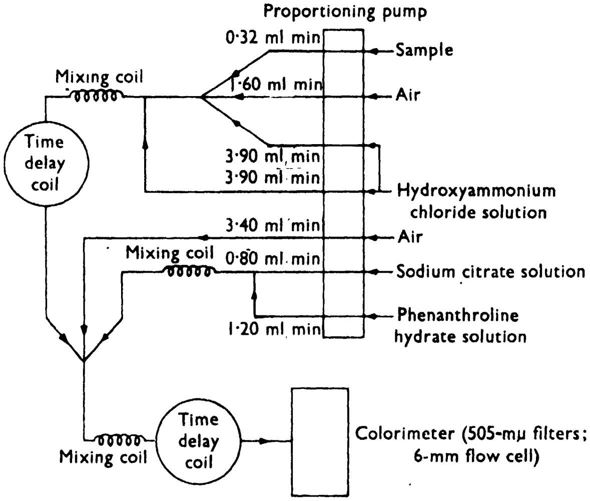

Fig. 4. AutoAnalyzer flow system for determining total iron in steel-making slags

RESULTS, PRECISION AND ACCURACY

The instrumental precision of the AutoAnalyzer system was determined by making 12 successive determinations on three solutions prepared from samples selected to give different optical-density values. Table II indicates that instrumental precision is related to optical density and varies from about 2 per cent. (as coefficient of variation) at the 0·05 level of optical density to about 0·3 per cent. at the 0·5 level. These errors do not include minor calibration errors that might be experienced in day-to-day operation or chemical errors involved in the preparation of the sample solution.

To measure the over-all analytical precision, six samples of British Chemical Standard No. 174/1 basic slag were analysed six times over a period of 3 months by the AutoAnalyzer procedures. These same sample solutions were also analysed by the manual methods; the individual results obtained are shown in part of Table IV, and the precision obtained with both manual and automatic procedures is compared in Table III. Over-all precision with the AutoAnalyzer is at least as good as that obtained by carefully operated manual processes and in the determination of the alumina it is noticeably better. This is probably due to the simpler procedure developed for automatic operation, which avoids the need for chemical separation to remove interfering elements.

The results obtained for the sample of standard slag (see Table III) are in close agreement with Certificate values. As an additional check on the accuracy of the automated methods, results obtained on a series of 10 duplicated sample solutions were compared with results obtained by the manual methods. The results are presented in Table IV together with results for the sample of standard slag mentioned above.

TABLE II

PRECISION OF THE INSTRUMENT

Sample	Mean optical density	Percentage equivalent, %	Standard deviation, %	Coefficient of variation, %
Manganese oxide—				
Slag "10"* (~ 1·75 per cent.)	0·0495	1·81	0·015	0·84
B.C.S. 174/1 (5·11 per cent.) ..	0·165	4·96	0·025	0·50
Slag "6" (~ 12·5 per cent.) ..	0·380	12·61	0·059	0·47
Alumina —				
B.C.S. 174/1† (1·82 per cent.) ..	0·035	1·71	0·050	2·9
Slag "10"† (~ 5·7 per cent.) ..	0·195	5·68	0·063	1·1
Slag "6" (~ 7 per cent.)	0·255	7·16	0·073	1·0
Total iron—				
Slag "6" (~ 2·5 per cent.)	0·112	2·54	0·014	0·56
B.C.S. 174/1 (8·45 per cent.) ..	0·400	8·48	0·023	0·27
Slag "10" (~ 15 per cent.)	0·659	14·91	0·034	0·23
Phosphorus pentoxide—				
Slag "6" (~ 1 per cent.)	0·031	1·00	0·020	2·00
B.C.S. 174/1 (12·3 per cent.) ..	0·374	12·20	0·060	0·49
Slag "10" (~ 15 per cent.)	0·457	14·89	0·054	0·36

* Range expansion × 4.
† Range expansion × 2.

TABLE III

COMPARISON OF PRECISION OBTAINED WITH AUTOANALYZER AND MANUAL METHODS FOR ANALYSING B.C.S. 174/1 BASIC SLAG

		Precision obtained with AutoAnalyzer			Precision of manual method		
	Certificate value and range, %	Mean of results, %	Standard deviation, %	Coefficient of variation, %	Mean of results, %	Standard deviation, %	Coefficient of variation, %
Manganese oxide	5·11 (5·00 to 5·15)	4·93	0·025	0·51	4·95	0·045	0·91
Total iron	8·45*	8·55	0·10	1·2	8·41	0·12	1·4
Phosphorus pentoxide	12·30 (12·16 to 12·50)	12·17	0·10	0·98	12·23	0·10	0·82
Alumina	1·82 (1·78 to 1·92)†	1·76	0·07	4·0	1·69	0·12	7·1

* Sum of mean results quoted for FeO and Fe_2O_3.
† B.I.S.R.A. Methods of Analysis Committee's results.

To establish whether or not a bias existed between the two procedures, the average discrepancy between the results was calculated, together with standard deviation and 95 per cent. confidence limits.

There is evidence of a positive bias in the determination of total iron, which is confirmed by the slightly higher results obtained for B.C.S. 174/1 standard slag (see Table III). Consideration of the manual method suggests that this bias and the high standard deviation of the average discrepancy may be due to errors introduced into the manual method during the

TABLE IV

COMPARATIVE RESULTS OBTAINED BY THE MANUAL AND AUTOMATIC COLORIMETRIC PROCEDURES

Sample number	Phosphorus pentoxide found			Total iron found			Manganese oxide found			Alumina found		
	By manual procedure, %	By Auto-Analyzer, %	Dif-ference, %	By manual procedure, %	By Auto-Analyzer, %	Dif-ference, %	By manual procedure, %	By Auto-Analyzer, %	Dif-ference, %	By manual procedure, %	By Auto-Analyzer, %	Dif-ference, %
Blast furnace slags—												
M.G.S. 408 ..	0·20	0·15	−0·05	0·40	0·25	−0·15	1·30	1·35	+0·05	14·5	14·2	−0·3
	0·25	0·15	−0·10	0·40	0·30	−0·10	1·40	1·30	−0·10	14·5	14·4	−0·1
M.G.S. 409 ..	0·10	<0·1	—	0·40	0·30	−0·10	0·95	0·95	0·00	14·8	14·7	−0·1
	0·05	<0·1	—	0·40	0·30	−0·10	0·95	0·95	0·00	15·0	14·7	−0·3
Basic arc furnace slags—												
M.G.S. 406 ..	1·00	1·05	+0·05	20·8	20·6	−0·20	9·15	9·30	+0·15	5·70	5·35	−0·35
	0·95	1·00	+0·05	20·6	20·6	0·00	9·00	9·20	+0·20	5·75	5·45	−0·30
B.I.S.R.A. 1 ..	0·35	0·40	+0·05	7·40	7·35	−0·05	4·45	4·35	−0·10	3·65	3·85	+0·20
	0·40	0·45	+0·05	7·40	7·55	+0·15	4·55	4·35	−0·20	3·65	3·75	+0·10
B.I.S.R.A. 2 ..	0·55	0·50	−0·05	7·45	7·60	+0·15	4·80	4·70	−0·10	3·45	3·40	−0·05
	0·60	0·50	−0·10	7·55	7·70	+0·15	4·75	4·65	−0·10	3·50	3·45	−0·05
B.I.S.R.A. 3 ..	0·85	1·00	+0·15	16·8	17·2	+0·40	10·9	10·8	−0·10	2·70	2·80	+0·10
	1·00	1·05	+0·05	16·8	17·2	+0·40	10·8	10·7	−0·10	2·65	2·75	+0·10
B.I.S.R.A. 4 ..	1·15	1·10	−0·05	20·2	20·6	+0·40	12·4	12·1	−0·30	2·50	2·15	−0·35
	1·15	1·10	−0·05	20·1	20·5	+0·40	12·4	12·4	0·00	2·35	2·05	−0·30
B.I.S.R.A. 5 ..	1·10	1·05	−0·05	24·0	24·2	+0·20	13·1	12·9	−0·20	1·65	2·00	+0·35
	1·20	1·10	−0·10	24·4	24·5	+0·10	13·2	12·9	−0·30	1·75	2·00	+0·25
B.I.S.R.A. 6 ..	1·40	1·35	−0·05	18·7	19·1	+0·40	12·5	12·6	+0·10	2·00	2·10	+0·10
	1·40	1·40	0·00	18·9	19·3	+0·40	12·4	12·3	−0·10	1·95	2·05	+0·10
B.I.S.R.A. 7 ..	0·15	0·15	0·00	1·05	1·10	+0·05	0·80	0·60	−0·20	1·45	1·55	+0·10
	0·15	0·15	0·00	1·05	1·00	−0·05	0·85	0·55	−0·30	1·55	1·75	+0·20
B.I.S.R.A. 8 ..	0·25	0·20	−0·05	0·85	1·10	+0·25	0·95	1·00	+0·05	1·55	1·90	+0·35
	0·25	0·20	−0·05	0·85	1·00	+0·15	0·95	1·05	+0·10	1·65	1·90	+0·25
B.I.S.R.A. 9 ..	0·15	0·15	0·00	2·35	2·25	−0·10	0·65	0·60	−0·05	4·10	4·40	+0·30
	0·15	0·15	0·00	2·30	2·15	−0·15	0·65	0·70	+0·05	4·25	4·45	+0·20

TABLE IV—*continued*

Sample number	Phosphorus pentoxide found			Total iron found			Manganese oxide found			Alumina found		
	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %
Basic open hearth furnace slags—												
M.G.S. 401 ..	17·1	17·0	−0·10	11·7	11·5	−0·20	3·40	3·40	0·00	0·45	0·50	+0·05
	17·1	17·1	0·00	11·8	11·8	0·00	3·45	3·40	−0·05	0·50	0·50	0·00
M.G.S. 402 ..	4·35	4·60	+0·25	24·9	25·1	+0·20	5·05	5·00	−0·05	1·70	1·70	0·00
	4·30	4·55	+0·25	24·9	25·2	+0·10	5·05	5·00	−0·05	1·80	1·85	+0·05
M.G.S. 403 ..	5·90	5·85	−0·05	11·3	11·0	−0·30	5·40	5·45	+0·05	2·95	2·75	−0·20
	5·85	5·75	−0·10	11·4	11·2	−0·20	5·40	5·45	+0·05	2·95	2·75	−0·20
M.G.S. 410 ..	9·90	9·85	−0·05	14·4	14·2	−0·20	14·3	14·2	−0·10	1·80	1·55	−0·25
	10·0	9·90	−0·10	14·5	14·3	−0·20	14·3	14·2	−0·10	1·90	1·60	−0·30
M.G.S. 411 ..	6·75	6·80	+0·05	12·6	12·6	0·00	10·5	10·4	−0·10	3·15	3·15	0·00
	6·85	6·80	−0·05	12·6	12·4	−0·20	10·5	10·4	−0·10	3·10	3·10	0·00
M.G.S. 412 ..	4·35	4·65	+0·30	29·4	29·1	−0·30	2·00	2·05	+0·05	0·75	0·75	0·00
	4·40	4·70	+0·30	29·4	29·3	−0·10	2·00	2·00	0·00	0·80	0·80	0·00
M.G.S. 413 ..	7·35	7·55	+0·20	4·50	4·60	+0·10	4·65	4·70	+0·05	1·80	1·80	0·00
	7·25	7·50	+0·25	4·50	4·55	+0·05	4·70	4·70	0·00	1·75	1·80	+0·05
B.C.S. 174/1	12·4	12·2	−0·02	8·35	8·60	+0·25	5·00	4·95	−0·05	1·80	1·85	+0·05
	12·2	12·2	0·00	8·40	8·55	+0·15	5·00	4·95	−0·05	1·75	1·85	+0·10
	12·1	12·0	−0·10	8·25	8·45	+0·20	4·90	4·90	0·00	1·70	1·70	0·00
	12·2	12·2	0·00	8·40	8·65	+0·25	4·90	4·95	+0·05	1·55	1·70	+0·15
	12·3	12·1	−0·20	8·60	8·60	0·00	4·95	4·90	−0·05	1·55	1·75	+0·20
	12·2	12·3	+0·10	8·45	8·40	−0·05	4·95	4·90	−0·05	1·80	1·70	−0·10
Average discrepancy and confidence limits ..			+0·01 ± 0·04			+0·05 ± 0·06			−0·05 ± 0·03			0·00 ± 0·06
Standard deviation of average discrepancy ..			0·12			0·20			0·12			0·19

transfer of small portions (1 or 2 ml) of the sample solution when the iron is present in high concentrations, *i.e.*, more than 10 per cent. Errors involved in volumetric transfer are eliminated in the automated procedure.

Results for manganese oxide show a small negative bias, but it is difficult to determine whether or not this is of practical significance.

There is no evidence of significant bias in the determination of phosphorus pentoxide and alumina. A high standard deviation of the average discrepancy was observed in the comparison of the alumina results, and this may possibly reflect errors introduced into the manual procedure by the chemical separation, as mentioned above.

CONCLUSIONS

The AutoAnalyzer system of automatic colorimetric analysis can be used for determining the total iron and the oxides of phosphorus, manganese and aluminium in iron- and steel-making slag. Accuracy and precision are equal to and possibly better than those obtained by carefully operated manual procedures.

After sample preparation and manual determination of silica, the time required for each of the automated procedures is 1 hour for the analysis of either 20 or 40 samples, depending on the type of chemical reaction involved. To this time must be added a period of about 30 to 40 minutes for assembling the apparatus, the passage of the first test solution through the flow system and the measurement of calibration and reagent blank solutions. After assembly and calibration of the apparatus, operation is completely automatic apart from the filling of sample-cups. While the instrument is in automatic operation, the operator can prepare additional sample solutions and convert optical-density readings to percentage concentration.

Recent developments in the AutoAnalyzer system have now made it possible to perform multiple analytical determinations. One sampler unit is coupled to a series of separate flow systems, each with its own colorimeter and chart recorder. Thus, if a combined system of this type were used, it would be possible to determine the four slag constituents simultaneously with a further saving in time.

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Analysis of Aluminium Alkyls

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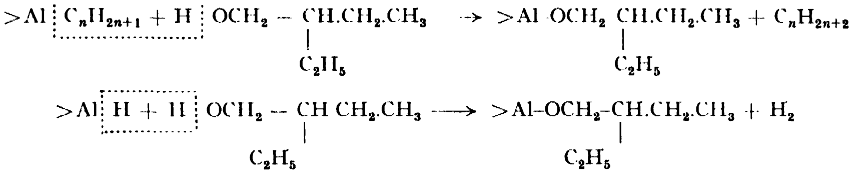
Organo-aluminium compounds, containing alkyl groups between methyl and butyl, are now used extensively as catalysts in processes for the manufacture of polyethylene and polypropylene, and are also of interest as intermediates in the manufacture of other chemicals. Trialkylaluminium compounds, dialkylaluminium chlorides or dialkylaluminium alkoxides containing these alkyl groups are all used as polymerisation catalysts. As well as alkyl groups these compounds frequently contain different proportions of aluminium-bound hydride groups produced during manufacture. Procedures are described for determining such hydride and alkyl groups in organo-aluminium compounds.

Bonitz[1] and Ziegler *et al.*[2] have developed gasometric procedures for the analysis of the lower aluminium alkyls, such as triethyl-, tripropyl- or tributylaluminium. In these procedures a known weight of sample reacts at a low temperature with an alcohol of high boiling-point, such as 2-ethylhexanol, under an atmosphere of nitrogen or helium. On alcoholysis each alkyl group liberates one mole of gaseous alkane and each hydride group liberates one mole of hydrogen.



$$>\mathrm{Al}\boxed{C_nH_{2n+1} + H}OCH_2-\underset{\displaystyle C_2H_5}{\underset{|}{CH}}.CH_2.CH_3 \rightarrow >\mathrm{Al}\ OCH_2\ \underset{\displaystyle C_2H_5}{\underset{|}{CH}}.CH_2.CH_3 + C_nH_{2n+2}$$

$$>\mathrm{Al}\boxed{H + H}OCH_2-\underset{\displaystyle C_2H_5}{\underset{|}{CH}}\,CH_2.CH_3 \longrightarrow >\mathrm{Al}\text{-}OCH_2\text{-}\underset{\displaystyle C_2H_5}{\underset{|}{CH}}.CH_2.CH_3 + H_2$$

The alkyl and hydride contents of the samples are then calculated from the amount of the gas evolved and its composition. Ziegler *et al.*[2] employed mass spectrometric and other methods of gas analysis for determining the composition of the gas mixture evolved.

Ziegler *et al.*[2] state that recoveries of gas by their procedure were lower than expected from the composition of the samples analysed. They attributed low gas yields to a partial dissolution of the evolved paraffin - hydrogen mixture in the 2-ethylhexanol reagent. We analysed several carefully purified samples of triethylaluminium and tri-isobutylaluminium and confirmed that recoveries of gas were lower than expected when 2-ethylhexanol or n-hexanol was used as the alcoholysis reagent.

From the results obtained in this work we considered it probable, however, that the low recoveries of gas obtained were due to incomplete reaction of alkyl and hydride groups with the alcoholic reagent, rather than dissolution of gas in the reagent. Thus, appreciably higher gas yields were obtained when sample decomposition was effected with a mixture of n-hexanol and monoethylene glycol or a mixture of water and monoethylene glycol than when 2-ethylhexanol was used alone. We therefore studied the reaction of lower alkyl groups (up to butyl) and hydride groups with a range of hydroxylic compounds (alcohols, glycol, water) in order to discover suitable reagents for the quantitative decomposition of each type of organo-aluminium compound.

Gas - liquid chromatography offered a simple and rapid method for analysing the gaseous mixture of alkane, hydrogen and nitrogen withdrawn from the gasometric apparatus after decomposition of the sample. We used this method of analysis throughout the investigation.

Work carried out to permit selection of suitable reagents for the determination of alkyl and hydride groups in trimethylaluminium, triethylaluminium, tri-n-propylaluminium, tri-isobutylaluminium and some of their chloro- and alkoxide derivatives is described below. A description is also given of the apparatus and procedure used in carrying out the analysis.

SELECTION OF HYDROXYLIC REAGENT

METHYLALUMINIUM AND ETHYLALUMINIUM COMPOUNDS—

It has been stated already that incomplete decomposition of the alkyl and hydride groups in triethylaluminium samples appeared to occur on reaction with 2-ethylhexanol or n-hexanol. Alternative reagents that would react quantitatively and smoothly with triethylaluminium were therefore sought.

We found that higher gas yields were obtained when a 4 + 1 mixture of n-hexanol and monoethylene glycol was used for decomposing triethylaluminium instead of anhydrous n-hexanol alone. It was decided, therefore, to see if a still higher yield of gas was obtained when an aqueous reagent was used for decomposing the sample.

It is not practicable to add water directly to triethylaluminium, diethylaluminium chloride or diethylaluminium ethoxide, as the ensuing reaction is extremely vigorous, even when carried out at −70° C. Also, we showed that an undesirable "fissioning" side reaction, which converts alkyl groups to ethylene and hydrogen instead of ethane, occurs to some extent when aqueous reagents or aqueous monoethylene glycol reagents are added directly to neat organo-aluminium compounds of low molecular weight.

Normal reaction— $> AlC_2H_5 + H_2O \longrightarrow C_2H_6 + {>}AlOH$

"Fissioning" reaction— $> AlC_2H_5 + H_2O \longrightarrow C_2H_4 + H_2 + {>}AlOH$

We found it possible to obtain complete reaction without any "fissioning" side reaction by using a combination of n-hexanol and aqueous reagents. The major decomposition of the alkyl is effected by an initial reaction with n-hexanol. An aqueous solution, containing 20 per cent. of sulphuric acid, is then added, and complete reaction occurs without "fissioning." No ethylene was detected in the gases liberated in these reactions.

Results obtained on typical samples of distilled trimethylaluminium and triethylaluminium are shown in Table I; it can be seen that yields of gas were good.

TABLE I

ANALYSIS OF FRESHLY DISTILLED METHYL AND ETHYL COMPOUNDS

Trimethylaluminium—	Content found, % w/w	
$Al(CH_3)_3$	99·5	99·2
$Al(CH_3)_2H$	Nil	Nil
Total	99·5	99·2

Triethylaluminium—	Content found, % w/w	
$Al(C_2H_5)_3$	94·4	94·6
$Al(C_2H_5)_2H$	2·2	2·2
$Al(C_2H_5)_2(C_4H_9)$	2·0	2·1
Total	98·6	98·9

Hydrolysis of chloro- or alkoxide derivatives results in the formation of hydrochloric acid or the corresponding alcohol; however, these compounds dissolve in the reagent, and we have shown that they do not interfere in the determination of alkyl groups.

PROPYLALUMINIUM COMPOUNDS—

Propylaluminium compounds are less reactive than are the ethyl compounds, and it was considered possible that the "fissioning" side reaction might not occur when a reagent containing water was added directly to the propyl compounds to which hexanol had not been added.

The first reagent tried was a 3 + 7 v/v mixture of monoethylene glycol and water; it was added to a sample of di-n-propylaluminium isopropoxide cooled to −30° C. Analysis of the gas obtained, however, showed the presence of considerable amounts of hydrogen and propylene, indicating that extensive "fissioning" of the propyl groups to hydrogen and propylene had occurred under these conditions.

$$>Al{-}CH_2{-}CH_2{-}CH_3 + H_2O \longrightarrow {>}AlOH + CH_3{-}CH{=}CH_2 + H_2.$$

We next tried a 3 + 7 v/v mixture of monoethylene glycol and 20 per cent. aqueous sulphuric acid. Rather surprisingly, propylene was completely absent from the gas generated in these experiments, indicating that "fissioning" did not occur when the aqueous reagent used was acidic. This decomposition procedure was then applied to the analysis of a sample of di-n-propylaluminium isopropoxide and also to a sample of tri-n-propylaluminium. The results obtained in the determination of propyl and hydride groups, together with separate determination of aluminium and n-propoxide[3] are shown in Table II.

TABLE II

ANALYSIS OF PROPYLALUMINIUM COMPOUNDS

Sample No.	n-Propyl found, % w/w	Hydride found, % w/w	n-Propoxide found, % w/w	Aluminium found, % w/w	Total of components, %	Empirical formula	Sum of subscripts in empirical formula	Departure of sum from 3·00 (*i.e.*, stoicheiometric AlR_3), %
Di-n-propylaluminium isopropoxide—								
1	48·8	<0·01	30·1	14·7	93·6	$Al_{1\cdot00}Pr_{2\cdot08}(OPr)_{0\cdot94}$	3·02	+1
2	43·3	<0·01	38·1	14·8	96·2	$Al_{1\cdot00}Pr_{1\cdot84}(OPr)_{1\cdot18}$	3·02	+1
Tri-n-propylaluminium—								
3	78·7	0·01	0·7	16·7	96·1	$Al_{1\cdot00}Pr_{2\cdot96}H_{0\cdot02}(OPr)_{0\cdot02}$	3·00	0
4	78·0	0·01	0·6	16·5	95·1	$Al_{1\cdot00}Pr_{2\cdot97}H_{0\cdot01}(OPr)_{0\cdot02}$	3·00	0

The total of the constituents determined was about 95 per cent. As it was known that these samples contained a few per cent. of hydrocarbon solvent, it was not possible to check the recovery of gas directly. Aluminium is always tervalent, however, and a test of the reliability of the analytical results is obtained when the calculated valencies, based on the analytical results, are compared with the value of 3. Values for the valency of aluminium calculated in this way are shown in Table II; it can be seen that they are within 1 per cent. of the theoretical value of 3. The recoveries of gas were therefore good.

A 3 + 7 v/v mixture of monoethylene glycol and 20 per cent. aqueous sulphuric acid was therefore adopted as a suitable reagent for the direct decomposition of propylaluminium compounds.

BUTYLALUMINIUM COMPOUNDS—

The two types of reagent developed for the ethylaluminium and the propylaluminium compounds were then applied to the analysis of butylaluminium compounds. The acidic glycol reagent was found to produce some "fissioning" of the butyl groups, which was overcome when the decomposition was carried out at −65° C, and recoveries of gas were slightly higher than with the n-hexanol - aqueous sulphuric acid reagent.

The results obtained on a sample of tri-isobutylaluminium are shown in Table III. In this sample the isobutoxide group content was determined by the method previously described.[3] It can be seen that the total of the components determined is close to 100 per cent., indicating good recovery of gas in the decomposition procedure.

TABLE III

ANALYSIS OF TRI-ISOBUTYLALUMINIUM

Constituent determined	Content found, % w/w	
$Al(C_4H_9)_3$	87·6	87·1
$Al(C_4H_9)_2H$	6·6	6·6
$Al(C_4H_9)_2(OC_4H_9)$	6·0	6·0
Total	100·2	99·7

LOWER ALKYL AND HYDRIDE GROUPS IN HIGHER ALKYLALUMINIUM COMPOUNDS—

It is sometimes necessary to determine alkyl groups, up to butyl, and hydride groups in organo-aluminium compounds containing alkyl groups higher than butyl. Higher molecular-weight organo-aluminium compounds can be prepared by the displacement of butyl groups in tri-isobutylaluminium with the appropriate higher olefin. These higher molecular-weight aluminium alkyls may still contain small amounts of hydride and lower alkyl groups.

The aqueous sulphuric acid - monoethylene glycol reagent, as used for the analysis of butylaluminiums, was found to be suitable for the analysis of these compounds. These materials were often highly viscous, and good recoveries of gas were obtained only when good mixing during the reaction was maintained by means of magnetic stirring.

METHOD FOR DECOMPOSING ALKYLS AND DETERMINING THE GASES EVOLVED

APPARATUS—

The apparatus, which is similar to that described by Ziegler *et al.*,[2] is shown in Fig. 1. It consists of a reaction vessel attached by means of a flexible coupling to a gas manifold system, incorporating a gas burette for volumetric measurement of the gas evolved, and leads to a gas sampling torpedo for transfer of the gas to the gas - liquid chromatograph for subsequent analysis. Mercury is used as the confining liquid in both the gas burette and in the sampling torpedo. The manifold system connects to a supply of pure nitrogen (white spot), which is dried by passage through a drying tower packed with Linde 4A molecular sieve.

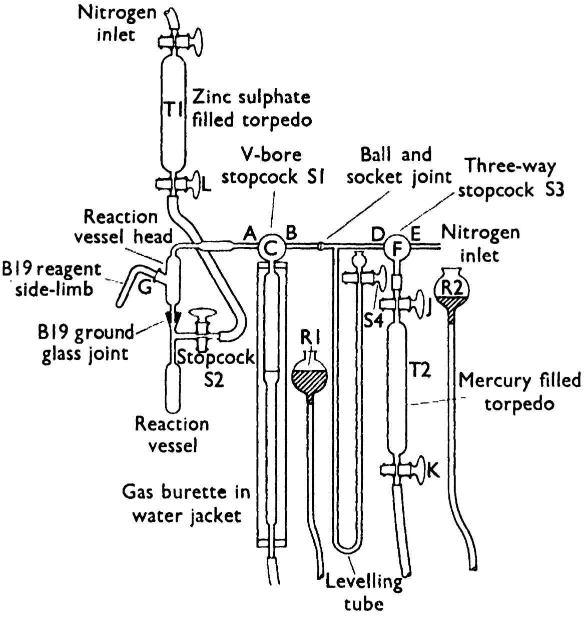


Fig. 1. Hydrolysis alcoholysis apparatus

The reaction vessel connects, via a stopcock, to a supply of aqueous zinc sulphate solution, which is used for discharging the reaction gases into the sampling torpedo.

The apparatus should be cleaned and dried thoroughly before each determination, and the cones at G and H and stopcocks S_1, S_2 and S_3 should be lubricated with silicone grease.

REAGENTS—

Decomposition reagent A—Mix 30 ml of 20 per cent. v/v aqueous sulphuric acid with 70 ml of monoethylene glycol. To 100 ml of mixed reagent add 1 ml of a non-ionic surface-active agent, *e.g.*, Nonidet P42, a condensation product of dioctyl phenol and ethylene oxide obtainable from the British Drug Houses Limited.

Decomposition reagent B—n-Hexanol.

Decomposition reagent C—Aqueous sulphuric acid (20 per cent. v/v) containing 0·05 per cent. of water-soluble methyl orange.

SAMPLING—

Organo-aluminium compounds received for analysis may contain a hydrocarbon diluent. Hydrocarbon diluents that boil below 180° C can be removed by vacuum distillation at a pressure of 0·1 mm of mercury at a maximum temperature of 65° C. Avoid heating above 65° C, as, at this pressure, many organo-aluminium compounds are somewhat volatile or may decompose above this temperature.

Transfer the appropriate weight of sample to the weighed reaction vessel by means of a safety pipette. Purge the reaction vessel and sampling pipette with dry nitrogen during transfer of the sample, as described by Crompton.[3]

The weight of an organo-aluminium compound required for a determination is that from which approximately 70 ml of gas at S.T.P. will be evolved. Calculate the weight of neat sample required from—

$$\text{Weight of sample} = \frac{70 \times 1000}{22400} \times \frac{M}{n} \text{ mg}$$

where M = molecular weight of the compound and
n = number of alkyl groups per molecule of compound.

Samples should be free from metallic sediment, such as free aluminium, as reaction of metal with the acid decomposition reagents would occur with the evolution of hydrogen, which would cause high results for aluminium-bound hydride. Metallic sediments can usually be completely removed from the sample by centrifugation.

PROCEDURE FOR PURGING THE APPARATUS—

At this stage the reagent side-limb, the reaction vessel and sampling torpedo T_2 are not connected to the apparatus. Open the gas burette to atmosphere, and raise mercury reservoir R_1 until the burette is filled with mercury to the barrel of stopcock S_1. Connect A to B with stopcock S_1, and purge with nitrogen through inlet I. Connect B and C, and allow the nitrogen pressure to depress the mercury until about 15 ml of nitrogen have entered the gas burette. Cut off the nitrogen pressure by connecting E to F with stopcock S_3. Lower reservoir R_1 until the mercury levels in the burette and reservoir are the same. Connect D to F with stopcock S_3, and then slowly raise reservoir R_1 until 5 ml of nitrogen remain in the burette. Connect A to B with stopcock S_1, and then D to E with stopcock S_3. Continue purging with nitrogen through inlet I.

DECOMPOSITION OF METHYL- AND ETHYLALUMINIUM—

By pipette place 1·5 ml of reagent C in the reagent side-limb, ensuring that no drops of this aqueous reagent remain above the liquid level. Then place from a pipette 1·5 ml of immiscible reagent B in the side-limb on top of the aqueous phase. This reagent, n-hexanol, will float on top of the aqueous phase, and no globules of aqueous reagent should be present in the upper hexanol layer. Connect the side-limb to the reaction vessel head, and connect springs across the glass lugs. Nitrogen now leaves via socket H.

Purge with nitrogen the interior of a clean oven-dried reaction vessel; use a glass inlet tube. Remove the inlet, and apply a gentle purge to the side-arm of the loosely stoppered reaction vessel. Discontinue the purge with nitrogen, close stopcock S_2, and closely stopper the vessel. Weigh the reaction vessel accurately. Purge the reaction vessel gently with nitrogen, remove the stopper, and, by pipette, place the required volume of sample in the reaction vessel. Stopper the vessel, close stopcock S_2, and re-weigh.

Remove the nitrogen supply line from inlet I, and immediately transfer it to the side-arm of the reaction vessel. Open stopcock S_2 on the reaction vessel, and then remove the stopper. Clamp the reaction vessel to the head of the apparatus, and close stopcock S_2. Fasten the springs connecting the reaction vessel to the head. Connect D to E with stopcock S_3, and rotate stopcock S_1 through one complete revolution to bring the internal pressure of the system to atmospheric pressure; connect A to C with stopcock S_1.

Adjust the height of mercury reservoir R_1 until the mercury level in both limbs of the U-levelling tube are the same, with stopcock S_4 open. Measure the volume of nitrogen in the burette, and record the atmospheric pressure and ambient temperature. The temperature of the water-jacket surrounding the gas burette should not differ by more than 1° C from room temperature when gas volumes are being read.

Immerse the lower bulb of the reaction vessel in a cooling bath maintained at −60° C. Leave for 5 minutes to cool, and level off the mercury in the gas burette and reservoir R_1 when necessary.

Slowly rotate the reagent side-limb until about half of the n-hexanol layer has flowed into the reaction vessel; ensure that none of the aqueous phase enters the reaction vessel at this stage. As generation of gas proceeds, equalise the mercury levels in the burette and reservoir R_1. When evolution of gas appears to be complete, remove the cooling bath, and allow the reaction vessel to attain room temperature. Immerse the reaction vessel in a cold-water bath, and heat up to 50° C. Add the aqueous reagent C by again slowly rotating the side-limb. Further evolution of gas occurs when this reagent is added, so continuously equalise the mercury levels in the gas burette and reservoir R_1. Heat the water-bath surrounding the reaction vessel to the boiling-point, and maintain at the boil for 30 minutes. Remove the water-bath, and again equalise the mercury levels as the gas contracts.

Allow the system to come to equilibrium overnight. Adjust the reservoir R_1 until the mercury levels in both limbs of the U levelling tube are the same, stopcock S_4 being open. Measure the volume of gas in the burette, and record the atmospheric pressure and room temperature.

Displace all the gas in the reaction vessel, etc., by first connecting mercury-filled sampling torpedo T_2, fitted with a reservoir, R_2, to inlet F on stopcock S_3. Connect E to F, and raise the mercury level to the barrel of stopcock S_3. Now connect D to F with stopcock S_3 and A to B with stopcock S_1. Close stopcock S_4 on the U levelling tube.

Now displace reaction gases by attaching a source of saturated zinc sulphate, supplied from a torpedo, T_1, to stopcock S_2. Open stopcocks S_2, L and M, and apply a gentle pressure of nitrogen at stopcock M. The zinc sulphate solution will now expel the gas contained in the reaction vessel and reagent side-limb. When the level of the zinc sulphate solution reaches A on stopcock S_1 close stopcock S_2, and connect B to C. Raise reservoir R_1 to displace the gas from the burette into torpedo T_2. Keep the mercury in torpedo T_2 and reservoir R_2 at the same level during transfer of the gas. Close stopcocks J and K, and disconnect the torpedo from the manifold. Shake the torpedo to mix the gas sample, and analyse the gas by gas - liquid chromatography.

TABLE IV

GAS - LIQUID CHROMATOGRAPHIC CONDITIONS

	Stage 1	Stage 2
	Hydrogen, methane and C_2 hydrocarbons	C_3 and C_4 hydrocarbons
Column	3 feet × $\frac{3}{16}$-inch i.d. glass	18 feet × $\frac{1}{8}$-inch stainless steel
Column packing	Davison silica gel 912, 28- to 60-mesh	12 feet of dimethylsulpholane + 6 feet of dinonyl phthalate (both 20 per cent. on 44- to 60-mesh Celite)
Column and detector temperature	30° C	30° C
Sample size	5 ml	0·25 ml
Detector	Katharometer	Katharometer
Bridge current	150 mA	150 mA
Carrier gas	Nitrogen (white spot)	Hydrogen
Carrier gas flow	2 litres per hour	3 litres per hour

DECOMPOSITION OF PROPYL- AND BUTYLALUMINIUM—

Carry out the decomposition of propyl- and butylaluminium compounds in the same way as described for the methyl- and ethylaluminiums, with the exception of the reagent composition. Use only one reagent, 2·5 ml of reagent A, in the reagent side-limb. Add this reagent, as before, by slowly rotating the reagent side-limb to give dropwise addition. During the warm-up period from —60° C to room temperature, however, the reaction may become vigorous. Should this happen, control it by temporarily returning the reaction vessel to the cooling bath. After reaction is complete, heat the water-bath surrounding the reaction vessel to the boiling-point, cool, and expel the gas into the mercury-filled torpedo, T_2, as previously described.

ANALYSIS OF EVOLVED GAS—

The gas-chromatographic analysis of the evolved gas is carried out in two stages. In the first stage hydrogen, methane and C_2 hydrocarbons are determined on a column of silica gel with nitrogen as carrier gas. In the second stage a combination of dimethylsulpholane and dinonyl phthalate columns is used for determining the C_3 and C_4 hydrocarbons.

Suitable gas - chromatographic conditions are shown in Table IV; any conventional gas chromatograph adapted for the analysis of gases can be used.

Calibrate the apparatus for hydrogen, methane and C_2 hydrocarbons by analysing accurately prepared mixtures of each gas with nitrogen. Determine the peak areas by electrical integration or from the product peak height × width at half peak height × attenuation factor.

The preparation of these calibration mixtures can be simplified by using a Wösthoff gas-blending pump (type A18/2a, obtainable from H. Wösthoff, O.H.G., Apparatbau, Bochum, Germany). Calibrate the gas - liquid chromatograph in terms of peak area against volume per cent. for hydrogen, methane and C_2 hydrocarbons; check this calibration periodically.

For the C_3 and C_4 hydrocarbons, use the gas-mixing pump to prepare mixtures of the gases with hydrogen, and determine the response factors for each gas relative to n-butane. This should be done accurately once every 4 months. Carry out a daily calibration check on a mixture of n-butane and hydrogen, and determine the area responses; the relative area response for each gas can then be determined for that day from the relative response factors. Calculate the percentage, by volume, of each gas from the product peak area × relative area response factor.

Calculate the total gas composition by normalising the individual percentage volumes so that their sum is 100 per cent.

CALCULATION OF RESULTS

CALCULATION OF GAS YIELD—

The volume of gas (V ml), corrected to S.T.P., generated during the reaction is given by—

$$V = \frac{273}{760} \cdot \left\{ \frac{(P_2 - P)(D + V_2)}{T_2} \quad \frac{(P_1)(D + V_1)}{T_1} \right\}$$

where D = volume (in millilitres) of dead space in apparatus, *i.e.*, the combined volume of the reaction vessel, side-limb and reaction vessel head. Determine D by weighing the amount of mercury needed to fill these three vessels.

P_1, P_2 = atmospheric pressure in mm of mercury when measuring initial and final gas volumes, respectively.

T_1, T_2 = ambient temperatures in degrees absolute when measuring initial and final gas volumes, respectively.

V_1, V_2 = volume of gas in millilitres in burette before and after evolution of gas, respectively.

P = a small correction term in mm of mercury allowing for the saturation vapour pressure exerted by the aqueous sulphuric acid - monoethylene glycol Reagent A. This correction term is sufficiently small to be ignored when the two phase n-hexanol - aqueous sulphuric acid reagent is used. The values of P at different temperatures are—

Temperature, °C	15	20	25	30
P, mm of mercury	6·9	9·4	12·7	16·9

CALCULATION OF ALKYL AND HYDRIDE CONTENTS—

These are given by—

$$\text{Hydride in sample} = \frac{a \times V \times 1{\cdot}008}{W \times 22{,}400}\ \%\ \text{w/w}$$

$$\text{Alkyl in sample} = \frac{b \times V \times M}{W \times C}\ \%\ \text{w/w}$$

where a = percentage by volume of hydrogen in generated gas.
b = percentage by volume of alkane in generated gas.
M = group weight of alkyl group being determined.
V = volume in millilitres of S.T.P. of gas generated in analysis.
W = weight in grams of organo-aluminium sample.
C = volume in millilitres per mole (at S.T.P.), relationship of the alkane gas involved, *e.g.*, for methane and ethane C = 22,400, for propane C = 21,970 and for butane C = 21,830.

CALCULATION OF COMPOUND COMPOSITION OF SAMPLE—

The method of calculating the compound composition of an organo-aluminium sample from its determined hydride, alkyl and alkoxide[3] contents is shown below. A, B, C and D are the percentage w/w contents of ethyl, hydride, ethoxide and butyl groups determined, respectively.

Then—

$$Al(C_2H_5)_2H = \frac{B \times (\text{mol. wt. of } Al(C_2H_5)_2H = 86{\cdot}1)}{(\text{atomic wt. of hydrogen} = 1{\cdot}008)}\ \%\ \text{w/w}$$

$$Al(C_2H_5)_2(OC_2H_5) = \frac{C \times (\text{mol. wt. of } Al(C_2H_5)_2(OC_2H_5) = 130{\cdot}15)}{(\text{group wt. of } (OC_2H_5) \text{ group} = 45{\cdot}06)}\ \%\text{w/w}$$

$$Al(C_2H_5)_2(C_4H_9) = \frac{D \times (\text{mol. wt. of } Al(C_2H_5)_2(C_4H_9) = 142{\cdot}20)}{(\text{group wt. of butyl group} = 57{\cdot}11)}\ \%\ \text{w/w}$$

$$Al(C_2H_5)_3 = \left\{A - \left(\frac{B}{1{\cdot}008} + \frac{C}{45{\cdot}06} + \frac{D}{57{\cdot}11}\right)2 \times 29{\cdot}06\right\}\frac{114{\cdot}15}{3 \times 29{\cdot}06}\ \%\ \text{w/w}$$

(where group weight of ethyl groups = 29·06 and molecular weight of triethylaluminium = 114·15).

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The Determination of Selenium in Biological Material by Radioactivation

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Neutron-activation analysis has been applied to the determination of selenium in biological material. Samples were activated in a flux of 10^{12} neutrons per sq. cm per second for 18 minutes, and a rapid chemical separation was developed for selenium-81. Under these conditions the practical limit of sensitivity was found to be 5×10^{-9} g. Decontamination from arsenic, bromine, manganese, sodium and zinc was tested and found to be satisfactory. Selenium contents of fertilisers, tomato tissue and human blood were measured by the procedure.

OUR knowledge of the behaviour of selenium in plants and animals has undoubtedly been hampered by the absence of sufficiently sensitive methods for determining the element. Until recently the only microanalytical method was that described by Robinson *et al.*,[1] which measures the red colour of elementary selenium after a chemical separation. With the discovery that trace amounts of selenium are important and possibly essential to mammals[2,3] there has been a revival of interest in other methods for determining the element. It appears that levels in normal biological tissue range from 0·005 to 0·5 µg per g so that only ultra-micromethods are suitable. Such methods include the combined spectrophotometric and isotope-dilution method proposed by Kelleher and Johnson[4] and Watkinson's fluorimetric method,[5] but most colorimetric methods[1,6,7] and X-ray fluorescence methods[8] are not yet sufficiently sensitive. To avoid errors due to reagent contamination, several workers have used neutron-activation techniques, and one such technique is described below.

Table I lists the radionuclides of selenium produced by thermal neutron activation and some of their relevant properties. It can be seen that only three of the nuclides can be made in high specific activities, and of these the best (selenium-77*m*) is exceedingly short-lived, with a half-life of only 17·5 seconds. Nevertheless, it has been used by several workers[9,10,11,12] for determining the element, with the use of an activation period of a few seconds and a multichannel analyser focussed on the 0·16-MeV gamma-ray peak. This can be a satisfactory method provided the half-life of the peak is measured, since many other short-lived nuclides have a gamma-ray of about the same energy.

TABLE I

RADIONUCLIDES PRODUCED BY A THERMAL NEUTRON FLUX OF 10^{12} neutrons per sq. cm per second ON SELENIUM

Radionuclide	Activity of selenium after activation for one half-life, mC per g	Half-life	Maximum beta energy, MeV	Gamma energies, MeV
Selenium-75	25	120 days	—	0·27 and 0·14
Selenium-77*m*	97	17·5 seconds	—	0·16
Selenium-79*m*	2·9	3·9 minutes	—	0·096
Selenium-81*m*	1·5	57 minutes	—	0·103
Selenium-81	25	18·6 minutes	1·60	none
Selenium-83*m*	0·46	69 seconds	3·40	1·01 and 2·02
Selenium-83	0·04	25 minutes	1·70	0·36 and 2·34

Most workers have used selenium-75 to determine the element.[13 to 19] This nuclide has a convenient gamma-ray for counting, and its half-life is more than adequate to allow complete chemical separation from other activities. Its main disadvantage is the long activation time needed. Most analysts have activated their samples for only 7 to 14 days, which gives them only 5 to 10 per cent. of the specific activity quoted in Table I. This results in an equivalent loss of sensitivity, which is undesirable for biological samples.

The use of shorter lived selenium isotopes for activation analysis has also been suggested. The group at the University of Michigan[20,21] have used a rapid chemical separation method coupled with gamma-ray spectrometry for detecting 3·9-minute selenium-79*m*, but the limit of detection is only 10 μg. Yajima *et al.*[22] have employed 57-minute selenium-81*m* for determining selenium in tellurium, but the sensitivity is also poor. In this work we have used 18-minute selenium-81, which has the advantage of giving a theoretical sensitivity equal to that obtainable by using selenium-75 and requiring a much shorter activation time. Selenium-81 is virtually a pure beta-emitter, which may explain why it has not previously been considered for activation work, though its use is mentioned by Leddicotte.[17]

METHOD

ACTIVATION—

Samples were collected under the cleanest possible conditions and sealed into clean polythene ampules. Standards were prepared by first dissolving selenium dioxide in water spotted on to weighed 1-cm × 1-cm squares of Whatman No. 541 filter-paper, which were then re-weighed. When dry, the standards were sealed in small polythene bags. The selenium content of these filter-papers was found to be $<0·01$ μg per square. For each run, four samples and two standards were packed into a plastic "Rabbit" and activated for 18 minutes in a flux of about 10^{12} neutrons per sq. cm per second in the Harwell reactor BEPO.

REAGENTS—

All reagents were of recognised analytical grade.

Ashing mixture—A (1 + 1) mixture, by volume, of 16 N nitric acid and 70 per cent. perchloric acid.

Nitric acid, 2 N.

Hydrochloric acid, 12 N.

Hydrobromic acid, 48 *per cent. w/v.*

Hydrogen peroxide, 30 *per cent. w/v.*

Teepol solution, 1 *per cent. v/v.*

Acetone.

Sulphur dioxide, liquefied.

Nitrogen, 99·9 *per cent. pure.*

Selenium carrier solution—Prepared by dissolving 2·8106 g of selenium dioxide in distilled water and diluting to 100 ml.

1 ml ≡ 20 mg of selenium.

Arsenic carrier solution—Sodium arsenate solution, 10 per cent. w/v.

Manganese carrier solution—Manganese nitrate solution, 50 per cent. w/v.

Phosphorus carrier solution—Ammonium dihydrogen orthophosphate solution, 10 per cent. w/v.

Tellurium carrier solution—Sodium tellurate, 10 per cent. w/v in N hydrochloric acid.

CHEMICAL SEPARATION OF SELENIUM—

Activated samples containing organic matter were placed in 150-ml beakers together with 10 ml of ashing mixture, 1 ml of selenium carrier solution and 2 drops each of arsenic, manganese, phosphorus and tellurium carrier solutions. They were boiled until the organic matter was destroyed and all nitric acid had volatilised, so that white fumes of perchloric acid were visible. They were then cooled and transferred to 50-ml round-bottomed flasks containing 5 ml of hydrochloric acid and 5 ml of hydrobromic acid. Distillation heads[23] were fitted immediately, and a current of nitrogen was passed through the flasks for approximately 4 minutes while they were heated with a small bunsen burner. The distillates were collected in 50-ml centrifuge tubes containing 5 ml of hydrochloric acid, 10 ml of water, 0·1 ml of Teepol solution and 2 drops of manganese carrier solution. Then sulphur dioxide was immediately passed into the centrifuge tubes through a glass capillary until a dark red precipitate of selenium was seen. This precipitate was separated by centrifugation, and dissolved in about 0·25 ml of nitric acid. Hydrogen peroxide (0·1 ml), 10 ml of hot water, and 5 ml of hydrochloric acid were added, and sulphur dioxide was again passed through the hot solution to precipitate selenium. This selenium was separated by centrifugation, washed with water and acetone and finally transferred to a weighed aluminium counting tray as a slurry with acetone. When dry, it was counted with the minimum of delay and was subsequently weighed. The mean chemical yield was 70 per cent., and most of the loss was mechanical.

Table II lists the time taken by these operations. In practice it was possible to begin counting two half-lives (36 minutes) after removal of six samples from the reactor, with two analysts performing the chemical manipulations.

TABLE II

TIME REQUIRED FOR UNIT PROCESSES IN THE CHEMICAL SEPARATION

Operation	Time required, minutes
Opening samples	1
Ashing	3
Distillation	4
First precipitation by sulphur dioxide	1
Centrifugation	1
Dissolution of selenium	0·5
Second precipitation by sulphur dioxide*	1
Centrifugation and washing	4
Transfer to counting tray	0·5
Drying	2

* This was the least reproducible step, and sometimes took up to 3 minutes.

TREATMENT OF STANDARDS—

The filter-paper standards were boiled for 4 minutes with 5 ml of hydrochloric acid, 10 ml of water, 0·1 ml of Teepol solution and 2 drops of manganese carrier solution. (Tracer experiments with selenium-75 showed that 99·7 per cent. of the selenium activity was recovered in this step.) The solutions were decanted into 40-ml centrifuge tubes, and sulphur dioxide was passed through the solutions. The selenium precipitates were washed and plated out as described above for the samples.

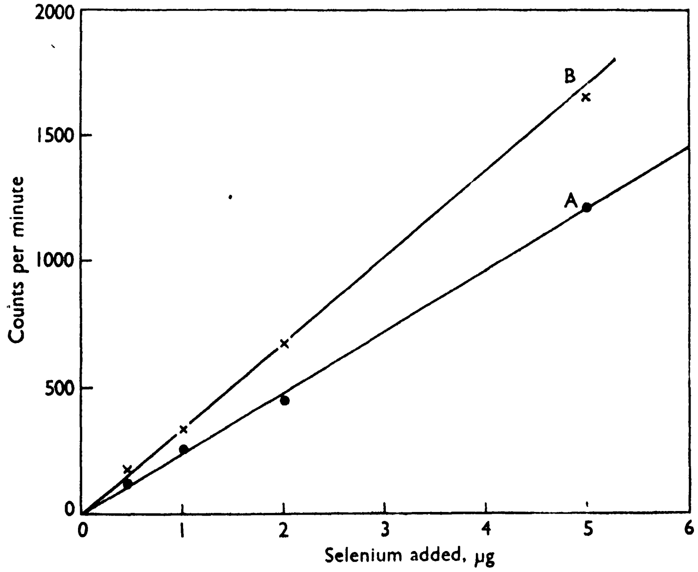

Fig. 1. Recovery of known amounts of selenium spotted on to: curve A, filter-paper; curve B, tomato seeds

DETERMINATION OF RADIOACTIVITY—

The beta-activity of the precipitates was counted with a 2B2 end-window Geiger counter of approximately 40 per cent. efficiency. Radiochemical purity was checked by comparing the decay curves of samples and standards over several half-lives. The decay curve of neutron-activated selenium is complex, since after the selenium-81 has largely decayed there are still counts from selenium-83, selenium-81*m* and selenium-75, which have longer half-lives (see Table I). Gamma spectrometry was also used for detecting possible impurities in precipitates having high count-rates.

DISCUSSION OF THE METHOD

Fig. 1 shows a recovery curve for known amounts of selenium spotted on to (*a*) filter-paper and (*b*) tomato seeds. Each point represents the mean of four determinations agreeing to ±5 per cent. It can be seen that the count rate is directly proportional to selenium content in the range 0·5 to 5 μg of selenium.

The theoretical sensitivity of the method is determined both by the flux available and by the background of the counter used. In our experiments, assuming a chemical separation time of 40 minutes and a background of 30 counts per minute, 5 μg of selenium in the sample would double this background. This amount can be taken as the practical limit of sensitivity when BEPO is used.

TESTING THE RADIOCHEMICAL PROCEDURES—

The chemical procedures described above were tested with portions of radiochemically pure arsenic-76, bromine-82, manganese-56, selenium-75, sodium-24 and zinc-65. The percentages of these nuclides contaminating the final precipitate and other fractions were determined by scintillation counting, and the results shown in Table III were obtained.

As might be expected, the volatile element bromine is largely eliminated during the initial ashing step, whereas the four metals remain in the residue after distillation. The amounts of arsenic, manganese, sodium and zinc found in the distillate must be a measure of the amount of spray carried over in our distillation procedure.

ACCURACY OF THE METHOD—

Errors may occur in the activation process, in the chemical processes, or during counting. The flux gradient in BEPO is less than 2 per cent. over the Rabbit volume. The high flux of fast neutrons in BEPO could give rise to the production of selenium-81 by the reactions—

(*i*) $^{81}\mathrm{Br}(n,p)^{81}\mathrm{Se}$.
(*ii*) $^{84}\mathrm{Kr}(n,\alpha)^{81}\mathrm{Se}$.
(*iii*) $^{235}\mathrm{U}(n,f)^{81}\mathrm{Se}$.

Little is known about reaction (*i*), though the cross-section for 14-MeV neutrons has been calculated to be 0·023 barns.[24] By activating ammonium bromide in BEPO we have shown that 1 g of bromine gives an apparent content of 22 μg of selenium. This is an upper limit since the ammonium bromide may have contained some selenium as an impurity. Since bromine is present in biological tissue in amounts ranging from 1 to 10 μg per g, the (n,p) reaction (*i*) should not give rise to a significant error.

Reaction (*ii*) can be neglected in biological material because of the extreme rarity of krypton and its low cross-section. For example, the normal concentration of krypton in blood is estimated to be only 0·0002 μg per ml, which is far below the concentration of selenium.[25]

Reaction (*iii*) can also be neglected since the fission yield of selenium-81 is only 0·13 per cent., and uranium is present in extremely small amounts in biological material, *e.g.*, 0·014 μg per ml of whole blood.[25]

As regards the chemical stages, the elements that are likely to distil under the conditions described here include antimony, arsenic, bromine, chlorine, germanium, iodine, mercury, tellurium and tin. Leddicotte[17] obtained excellent decontamination from antimony, arsenic, sodium, tellurium and tin, by using a similar but slightly simpler separation. The nuclides of arsenic, germanium, mercury and tin have very different half-lives from that of selenium-81, but interference could be serious from 21-minute antimony-124, 25-minute tellurium-131, 37-minute chlorine-138 or 18-minute bromine-80. In biological material we regard bromine-80 as the most serious impurity since it has the same half-life as selenium-81, and bromine is much more abundant than either antimony or tellurium. Fortunately, decontamination from the halogens is reasonably good, as shown in Table III. The only long-lived impurity we have been able to find in our samples was manganese-56. Because of the high cross-section of manganese, this nuclide constitutes a major source of radioactivity in activated vegetable material, and even after a distillation step traces may contaminate the final selenium precipitate. If hydrogen peroxide is added when the selenium precipitate is dissolved in nitric acid, we find that decontamination from manganese is markedly improved; acidified hydrogen peroxide is a well-known solvent for manganese dioxide.

TABLE III

PERCENTAGES OF SIX ELEMENTS FOUND IN RADIOCHEMICAL FRACTIONS

Fraction	Arsenic found, %	Bromine found, %	Manganese found, %	Sodium found, %	Zinc found, %	Selenium found, %
Volatilised during ashing ..	0	95·41	0	0	0	0·25
Distillation residue ..	99·30	0·04	99·88	99·74	99·78	0·95
First selenium supernate ..	0·69	4·54	0·12	0·26	0·14	0·55
Second selenium supernate	0·019	0·013	0·00005	0·0015	$\leqslant$0·05	0·55
Final selenium precipitate	0·00037	0·0027	0·00019	0·0000081	$\leqslant$0·03	97·70

TABLE IV

SELENIUM CONTENT OF VARIOUS MATERIALS

Sample	Selenium content
N. African phosphate rock..	10·6 µg per g
Superphosphate	3·77 µg per g
Tomato leaf	0·088 µg per g
Tomato seed	0·025 µg per g
Tomato leaf*	1·25 µg per g
Tomato fruit*	0·24 µg per g
Human blood, mean	0·32 µg per ml
Human blood, range of 8 samples ..	0·26 to 0·37 µg per ml

* Grown in soil rich in selenium.

A small error is introduced in weighing the final precipitate, since this only weighs about 15 mg and ordinary balances are reliable to ±0·2 mg.

When counting long-lived nuclides it is possible to obtain counting accuracy of within 1 per cent., by registering 10,000 counts. This may not be possible for rapidly decaying nuclides; a count rate of 10,000 counts per minute is given from samples containing about 1·5 µg of selenium, and many biological samples contain much less than this. Care must also be taken to record the exact time at which a count is begun, since an error of 1 minute corresponds to a 3·5 per cent. correction for radioactivity decay.

RESULTS

Some results obtained by this method of analysis are shown in Table IV. The results for plant tissues and blood are of the same order of magnitude as those determined by earlier workers.[5,10,25] The relatively high figures for the widely used fertilisers, phosphate rock and superphosphate, are a little disquieting in view of the toxicity of the element. Tagwerker[3] states that any soil containing more than 0·5 µg per g of selenium is potentially dangerous and that chronic selenium toxicity may be caused by rations containing more than 5 µg per g of selenium.

We thank Miss M. J. Dick for carrying out most of the counting involved in this work.

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Determination of Thorium and Phosphorus Pentoxide in Solution and in Insoluble Thorium Phosphate

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Methods are described for the gravimetric determination of thorium in the presence of substantial amounts of phosphate, and of phosphate in the presence of thorium. The application of these methods to the analysis of insoluble thorium phosphates is described.

IN the course of studies on the preparation of thorium phosphates[1] by solid-phase and precipitation reactions, a method for determining thorium and phosphate present together in solution or in an insoluble compound was needed. Accurate methods for determining thorium in its pure compounds or in the absence of interfering substances such as phosphate are readily found, but procedures for use in the presence of relatively high concentrations of phosphate have not been described.

Some excellent reviews[2,3,4] on the determination of thorium are too voluminous to be summarised here. Although thorium can be precipitated as thorium iodate[5,6] from nitric acid solution, and the separation can be achieved in the presence of phosphoric acid, the well-known method of determining thorium oxalate deserved attention in view of its simplicity. Accordingly, a procedure was established by which thorium could be determined by this method even in presence of a fairly large amount of phosphate.

In preliminary work an attempt was made to separate thorium from phosphate by adsorbing the thorium ions on a cation-exchange resin (Zeo-Karb 225), initially in the hydrogen form. Thorium was strongly adsorbed by the resin, but all attempts to elute it afterwards, even by strong mineral acids, failed. This was not unexpected in view of the high charge on the thorium ion, Th^{4+}, and hence the process was abandoned. The separation of thorium from phosphate by ion-exchange was apparently successful, but the quantitative recovery aimed at could not be achieved. Determination of phosphate was also attempted after separation of thorium on the same cation-exchange resin. The results were not reproducible, and about 3 per cent. of the total phosphate was lost on the resin column. Evidently some phosphate was retained in the form of a cation complex.

Schoeller and Powell's method[7] for determining phosphoric acid in monazite sand was examined to see whether or not it could be used in presence of large amounts of thorium. Twenty-five millilitres of phosphoric acid solution (0·0908 g of phosphorus pentoxide) and 25 ml of thorium nitrate solution (0·1081 g of thorium dioxide) were placed in a 250-ml beaker. The thorium phosphate jelly was dissolved by adding 2 to 3 ml of concentrated sulphuric acid. The solution was saturated with potassium sulphate and after thorough mixing was set aside overnight; the bulk of the thorium was precipitated as thorium sulphate.[7] After a single precipitation of thorium by this method the amount of phosphate eventually recovered was 3·9 to 4·1 per cent. less than that originally taken; after double precipitation the amount of phosphate recovered was 1·2 to 1·7 per cent. less than that taken. Schoeller and Powell's method, designed for use when only small amounts of thorium are involved, is evidently not successful when major amounts of thorium are present.

METHOD

REAGENTS—

Standard thorium nitrate solution—Prepared from AnalaR thorium nitrate. Thorium was determined as thorium oxide after precipitation by oxalate solution.[6] The standard solution gave 0·1081, 0·1083 and 0·1079 g of thorium dioxide in 25-ml portions.

Standard phosphoric acid solution—Prepared from AnalaR grade phosphoric acid.

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Phosphorus pentoxide was determined by double precipitation with magnesia mixture and ignition of the magnesium ammonium phosphate to magnesium pyrophosphate. Treatment of two 25-ml portions of standard solution gave 0·1424 and 0·1424 g of magnesium pyrophosphate (0·0908 and 0·0908 g of phosphorus pentoxide).

Oxalic acid wash liquid—A 2 per cent. aqueous solution of oxalic acid containing 1 ml of concentrated nitric acid per 100 ml of solution.

DETERMINATION OF THORIUM IN THE PRESENCE OF PHOSPHATE IN A SOLUTION—

Mix 25 ml of thorium nitrate solution and 25 ml of phosphoric acid solution in a 400-ml beaker. Dissolve the jelly formed by adding 10 ml of concentrated nitric acid and warming. Add about 25 ml of water, and then add, dropwise, to the boiling solution 30 to 40 ml of a saturated solution of oxalic acid, with constant stirring. Finally, dilute the solution with water to about 300 ml to reduce the acid concentration to the admissible maximum of 3·5 ml of concentrated nitric acid per 100 ml of solution. Never dilute the solution before adding oxalic acid solution; in the absence of oxalic acid the insoluble phosphate jelly reappears. Boil the diluted solution for about 5 minutes, and set aside overnight.

Filter the solution through a Whatman No. 40 filter-paper; allow most of the precipitate to remain in the beaker. Wash the beaker and the precipitate on the filter-paper with about 150 ml of oxalic acid wash liquid. Transfer the precipitate, with the filter-paper, to the original beaker containing about 100 ml of concentrated nitric acid, and add about 5 ml of concentrated hydrochloric acid. Break up the filter-paper, and gently boil the solution in the covered beaker. After about 45 minutes the solution should be clear brown and much of the filter-paper should have been destroyed. Continue boiling the solution until its volume is reduced to about 25 ml. Transfer the beaker to a steam-bath, cautiously evaporate some of the remaining acid, and, when crystals begin to appear, cool the beaker. (It is important at this stage not to evaporate to dryness, otherwise the products tend to become insoluble.) Wash the sides of the beaker and the clock glass with the minimum amount of water. Add about 20 ml of concentrated nitric acid, and evaporate the solution nearly (but not completely) to dryness on a steam-bath. Cool the solution, and then add about 2 ml of concentrated nitric acid and 20 ml of a saturated solution of oxalic acid; dilute the solution as described above, and set aside overnight. Filter the solution through a Whatman No. 40 filter-paper, and thoroughly wash the precipitate of thorium oxalate with oxalic acid wash liquid. Transfer the precipitate and paper to a platinum crucible, dry, and then char the paper over a low flame. Finally, convert the precipitate into thorium dioxide by ignition over a full flame until it is white in colour and constant in weight.

Thoria was satisfactorily determined by this procedure; 25-ml portions of thorium nitrate solution (0·1081 g of thoria) in the presence of 25-ml portions of phosphoric acid (0·0908 g of phosphorus pentoxide) gave the results tabulated below—

Thoria found, g	0·1082	0·1079	0·1080
Deviation, %	+0·09	−0·18	−0·09

DETERMINATION OF PHOSPHATE IN PRESENCE OF THORIUM IN SOLUTION—

It was eventually found that the most convenient method for determining phosphorus pentoxide in the presence of thorium was that reported by Hoffman and Lundell[8] for determining phosphorus pentoxide in phosphate rock. Satisfactory results were obtained only when precautions similar to those suggested by Hoffman and Lundell were taken—

(*a*) A large amount of citric acid must be used in order to keep the thorium in solution.

(*b*) A concentrated solution of "magnesia mixture" is necessary.

(*c*) Before precipitation of ammonium magnesium phosphate, it is necessary to chill the solution in ice for at least half an hour; after precipitation it must be vigorously shaken for 1 hour to break down super-saturation.

The results of determinations of phosphorus pentoxide under different conditions of precipitation are shown in Table I. It can seen that vigorous shaking during precipitation is essential if reproducible results are to be obtained.

TABLE I

THE EFFECT OF DIFFERENT CONDITIONS OF PRECIPITATION ON THE RECOVERY OF PHOSPHORUS PENTOXIDE

Conditions of precipitation	Thoria taken, g	Phosphorus pentoxide taken, g	Phosphorus pentoxide found, g	Deviation, %
Solution chilled in ice and shaken vigorously	0·1104	0·0933	0·0933	0·0
			0·0931	−0·2
			0·0936	+0·3
Precipitation at room temperature without shaking the solution	0·1104	0·0933	0·0914	−2·0
			0·0915	−1·9
Solution chilled in ice but not shaken	0·1104	0·0933	0·0921	−1·3
			0·0916	−1·8

DETERMINATION OF THORIUM AND PHOSPHORUS PENTOXIDE IN INSOLUBLE THORIUM PHOSPHATES—

Grind the thorium phosphate to a powder in an agate mortar, and weigh into a platinum crucible. Add AnalaR sodium carbonate (5 to 6 times the weight of the sample), and mix the powders thoroughly. Fuse the mixture over a bunsen burner for about half an hour, cool, and transfer the crucible to a 150-ml beaker. Cover the crucible with water, and place the beaker on a steam-bath for about an hour, to disintegrate completely the fused mass. Filter the liquid through a 9-cm Whatman No. 40 filter-paper after adding macerated paper. Wash the crucible, the beaker and the residue on the filter-paper with about 250 ml of a 1 per cent. solution of sodium carbonate, and collect the filtrate in a 500-ml conical flask. Acidify the filtrate with hydrochloric acid, and evaporate to about 75 ml.

Transfer the residue, with the filter-paper, to a platinum crucible; burn off the paper, and then ignite the residue gently for 10 minutes. Mix with AnalaR sodium carbonate (about five times the weight of the original specimen), and fuse the mixture for half an hour. Disintegrate the fused cake in water as described, and filter the solution through a 9-cm Whatman No. 40 filter-paper after adding macerated paper. Collect the filtrate and washings (about 200 ml of 1 per cent. ammonium chloride solution) in the original conical flask. Add more hydrochloric acid to keep the solution acidic, and reduce the volume to about 100 ml by boiling. Then determine phosphorus pentoxide by double precipitation with magnesia mixture by the conventional procedure.

Transfer the filter-paper containing the residue to a weighed silica crucible, ignite, and heat until the weight of ignition residue is constant. Then add powdered potassium pyrosulphate (ten times the weight of the residue), and fuse the mixture over a small bunsen flame for 15 to 20 minutes, or until the thoria has completely dissolved in the pyrosulphate melt.

Transfer the crucible and fused cake to a 400-ml beaker, and cover the crucible with about 150 ml of water and 5 ml of concentrated nitric acid. Heat on the steam-bath for half an hour, until the fused cake has disintegrated and much of it has dissolved. Stir the solution at frequent intervals while heating for a further half hour to complete dissolution. Remove and wash the crucible, and adjust the volume of the solution to about 200 ml. (Whenever an attempt was made to dissolve the fused cake by *boiling* with water, an insoluble crystalline precipitate resulted.) Boil the solution, and precipitate thorium oxalate by adding a saturated solution of oxalic acid, as described previously. From this stage the procedure corresponds exactly with that described for determining thorium in the presence of phosphate in solution. The results of several determinations on different synthetic specimens of thorium phosphate are presented in Table II.

It is noteworthy that the percentage of thorium oxide found after pyrosulphate fusion is always considerably less than the percentage of "ignited residue," and that the thoria *plus* phosphorus pentoxide percentage is substantially less than 100; the thoria *plus* ignited residue percentages, however, are closer to the expected values of very nearly 100. This suggested that the high concentrations of alkali-metal ions inevitably introduced by the fusion with pyrosulphate had interfered with the precipitation of thorium oxalate, and that the ignited residue values more nearly represent the thoria content of the samples. To elucidate this point and to exclude other sources of error (such as retention of thoria by slight reaction with the silica crucible), test determinations were carried out on pure thoria. Sodium pyrosulphate was used for some of the fusions, because of the possibility that sodium might cause less interference in the oxalate precipitation than potassium.

TABLE II

RESULTS

Weight of thorium phosphate taken, g	Ignited residue, %	Thoria found after pyrosulphate fusion, %	Phosphorus pentoxide found, %	Thoria + phosphorus pentoxide found, %	Ignited residue + phosphorus pentoxide found, %
0·5328	58·90	56·68	40·37	97·05	99·27
0·5100	74·27	73·04	25·43	98·47	99·70
0·4836	73·20	71·53	26·55	98·08	99·75
0·5362	71·54	70·53	27·83	98·36	99·37
0·3399	69·13	67·46	30·34	98·80	99·47
0·5464	66·86	65·57	32·41	97·88	99·27

The entire fusion process, starting with the double fusion with sodium carbonate, was carried out on pure thorium oxide, prepared by igniting AnalaR thorium nitrate at 600° C in a platinum dish for 5 to 6 hours. Determinations were carried out in which both silica and platinum crucibles were employed for the pyrosulphate fusion. The results are presented in Tables III and IV.

TABLE III

FUSION WITH POTASSIUM PYROSULPHATE IN SILICA CRUCIBLES

Weight of thoria taken, g	Weight of ignited residue, g	Thoria found after potassium pyrosulphate fusion, %	Loss in weight of silica crucible during fusion, g
0·3050	0·3054	97·34	0·0013
0·2924	0·2927	97·50	0·0019
0·3284	0·3282	97·75	0·0018
0·2953	0·2950	97·49	0·0016

TABLE IV

FUSION IN PLATINUM CRUCIBLES

Fusion reagent	Weight of thoria taken, g	Weight of ignited residue, g	Thoria found after pyrosulphate fusion, %
Potassium pyrosulphate	0·3232	0·3230	97·58
	0·2960	0·2963	97·09
	0·3046	0·3044	97·31
Sodium pyrosulphate	0·3023	0·3025	97·32
	0·3139	0·3142	97·74

These results show that the weight of thoria obtained after fusion with sodium or potassium pyrosulphate is always less by about 2·5 per cent. than the weight of thoria taken. Both sodium and potassium ions evidently have an adverse effect on the precipitation of thorium oxalate. The ignited residue, however, corresponds accurately in weight with the thoria initially taken.

Attempts to dissolve the ignited residue by heating with concentrated sulphuric acid, so that the introduction of alkali metals was avoided, were unsuccessful.

The insoluble thorium phosphates were therefore fused twice with sodium carbonate, as described, and phosphorus pentoxide was determined in the water extract by double precipitation with magnesia mixture. The ignited residue was taken to represent the thoria present in the original sample.

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SHORT PAPERS

A Field Method for determining 2,4-Tolylene Di-isocyanate Vapour in Air

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TOLYLENE DI-ISOCYANATE is widely used in the production of urethane products such as flexible foams, lacquers and synthetic rubbers. During the manufacture of these materials the vapour of tolylene di-isocyanate may be evolved, and this can constitute a danger to health if proper care in storage, handling and ventilation is not taken. The effectiveness of precautionary measures can best be assessed by measuring atmospheric concentrations of tolylene di-isocyanate at appropriate times and places.

The recent reduction in the maximum permissible concentration of tolylene di-isocyanate from 0·1 to 0·02 p.p.m. v/v in air[1,2] has meant that the widely used M.S.A. T.D.I. Detector is insufficiently sensitive for the determination of concentrations in the region of the new limit. With this detector, based on the work of Marcali,[3] a 3-litre sample of air is drawn through 15 ml of dilute hydrochloric acid, when tolylene di-isocyanate is hydrolysed to the corresponding diamine; this is then diazotised with a sodium nitrite - sodium bromide solution, the excess of nitrite removed by treatment with sulphamic acid and the diazonium compound coupled with *N*-1-naphthylethylenediamine to give a pink coloured solution. The colour of the solution is then matched visually in turn against three strips of pink coloured plastic material representing concentrations of 0·05, 0·10 and 0·20 p.p.m. of tolylene di-isocyanate, respectively. This method is claimed to determine primarily the 2,4-isomer, as the 2,6-isomer is stated to react much more slowly.

Concentrations in the region of 0·02 p.p.m. can be determined with the existing method by increasing the sample size from 3 to 15 litres, but this involves either an inconveniently long sampling time of 15 minutes or the use of a larger (but less portable) pump to sample at a faster rate. This paper describes a modification of the earlier method in which a 3-litre sample of air is drawn through a specially designed absorber containing 3 ml of a mixture of dilute hydrochloric acid and *N*,*N*di-methylformamide.[4] The pink colour is developed as described above and matched visually against inorganic colour standard solutions.

METHOD

APPARATUS—

Sampling pump—Capable of drawing air at a rate of 1 litre per minute through the absorber. The M.S.A. 4-cylinder hand-cranked pump has been found convenient for this purpose. Alternatively, when convenient, a water-filled aspirator may be used.

All-glass absorber—Of the type shown in Fig. 1. The dimensions of the narrow lower part are important and should be adhered to; the upper part should be sufficiently large to prevent loss of the contents of the absorber by splashing during sampling.

Test-tubes—100 mm × 10 mm.

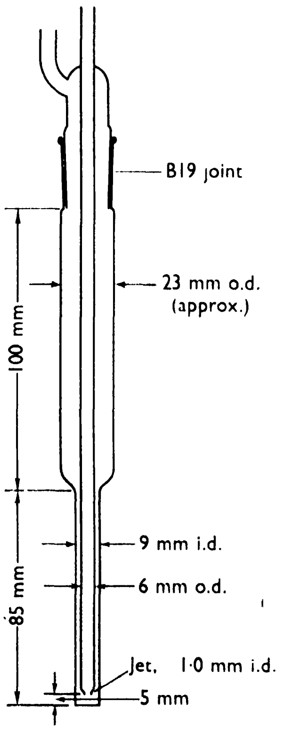

Fig. 1. All-glass absorber

REAGENTS—

Sodium nitrite - sodium bromide solution—Dissolve 3·0 g of sodium nitrite and 5·0 g of sodium bromide in about 80 ml of water, and dilute to 100 ml.

Sulphamic acid solution, 10 *per cent. w/v.*

Hydrochloric acid, dilute—Dilute 25 ml of concentrated hydrochloric acid, sp.gr. 1·18, to 1 litre with water.

N-1-*Naphthylethylenediamine dihydrochloride solution*—Dissolve 50 mg of the dry salt in about 25 ml of water, add 1 ml of concentrated hydrochloric acid, and dilute to 50 ml with water. Use within 48 hours.

N,N-*Dimethylformamide*—Distil through a water-cooled all-glass condenser, rejecting the first and last 10 per cent. of the distillate.

Absorber solution—Add 25 ml of concentrated hydrochloric acid, sp.gr. 1·18, to 500 ml of water. To this solution add 250 ml of *N*,*N*-dimethylformamide, and dilute to 1 litre with water.

Cobaltous chloride colorimetric solution—A solution in dilute hydrochloric acid containing 59·5 mg of the analytical-reagent grade salt, $CoCl_2.6H_2O$ (purity not less than 97·5 per cent.) per ml.[5]

Cupric sulphate colorimetric solution—A solution in dilute hydrochloric acid containing 62·4 mg of the analytical-reagent grade salt $CuSO_4.5H_2O$ (purity not less than 99·0 per cent.) per ml.[5]

Colour standard solutions—Mix the volumes of the cobaltous chloride and cupric sulphate colorimetric solutions specified in Table I, and dilute to 500 ml with dilute hydrochloric acid. These solutions have the same quality and depth of shade as the test solutions (containing the equivalent amounts of tolylene di-isocyanate) prepared as described below. Store in clean glass bottles having tightly fitting glass stoppers.

TABLE I

COMPOSITION OF COLOUR STANDARD SOLUTIONS

Cobaltous chloride solution, ml	Cupric sulphate solution, ml	Tolylene di-isocyanate, p.p.m.
8·75	13·75	0·01
17·50	27·50	0·02
35·00	55·00	0·04

PROCEDURE—

Draw 3 litres of the air sample at a rate of about 1 litre per minute through the absorber containing 3·0 ml of absorber solution. Disconnect and lift the inlet tube, allowing the liquid in it to drain into the body of the absorber. Expel the last drop by blowing gently on the inlet tube, and then withdraw this completely. Add 0·1 ml (3 drops) of sodium nitrite solution, close the absorber body with a glass stopper, and mix the solution by gentle shaking; set aside for 1½ minutes. Add 0·1 ml (3 drops) of sulphamic acid solution, stopper the absorber, mix the solution by gentle shaking, and set aside for 1½ minutes. Add 0·1 ml (3 drops) of *N*-1-naphthylethylenediamine dihydrochloride solution, stopper the absorber, and mix the solution by gentle shaking. Fill 100-mm × 10-mm test-tubes with the three colour standard solutions to the same depth as that of liquid in the absorber. Between 1½ and 2 minutes after adding the last reagent to the contents of the absorber, compare the colour of the test solution with each of the colour standard solutions in turn by looking downwards through the solutions towards a sheet of white paper held a few inches below the bottom of the tubes. Report the concentration of tolylene di-isocyanate as less than, equal to or more than that of the nearest colour standard.

BASIS OF METHOD

During the development of this method, a number of relevant factors were investigated.

STRENGTH OF COLOUR STANDARD SOLUTIONS—

The composition of these solutions was arrived at by a process of trial and error. The quality of shade was first matched by mixing cupric sulphate and cobaltous chloride solutions in different proportions until the shade of the mixture was judged to be correct by two people. Solutions of different strengths, but with the two components in the same proportion, were next prepared and matched with test solutions prepared from 0·21, 0·42 and 0·84 μg of tolylene di-isocyanate (corresponding to the weights present in 3 litres of test samples containing 0·01, 0·02 and 0·04 p.p.m. of tolylene di-isocyanate). When the approximate strengths of the inorganic standards had been ascertained, further solutions were prepared covering narrower concentration ranges, and the final concentrations were decided on.

In preparing coloured solutions from tolylene di-isocyanate, a standard solution containing 7·0 μg per ml was prepared in aqueous acetic acid as described by Marcali[3]; 0·5-, 1·0- and 2·0-ml portions of this solution were diluted to 50 ml with dilute hydrochloric acid, and 3·0-ml portions of these diluted solutions were then treated as described above.

Reaction with the 2,6-isomer—

Under the conditions described, the conclusion[2] that the reaction was specific for the 2,4-isomer was largely confirmed. There was a slow reaction with the 2,6-isomer, which produced a colour much bluer than that produced by the 2,4-isomer. The inorganic colour standards were matched against colours produced from a mixture containing 80 per cent. of the 2,4-isomer and 20 per cent. of the 2,6-isomer. The error when these standards were used for determining other commercial tolylene di-isocyanates, whose 2,4-isomer content might range from 60 to 100 per cent., would not be a serious one.

Time required for colour development—

In the procedure it is recommended that the matching of the colour of the test solutions with that of the inorganic colour standards be done between 1½ and 2 minutes after the addition of the *N*-1-naphthylethylenediamine dihydrochloride. This time interval was adopted because, on further standing, the colour of the test solutions deepened slightly and became bluer in shade, presumably owing to slow reaction of the 2,6-isomer.

Recovery from a sample of air—

No attempts were made to produce accurately known atmospheric concentrations of tolylene di-isocyanate. It was found, however, that concentrations in the range 0·01 to 0·10 p.p.m. were produced when a stream of nitrogen was bubbled through 20 ml of 2,4-tolylene di-isocyanate in a 15-cm × 2·5-cm test-tube at 20° C, at speeds from 10 to 100 ml per minute, and diluted with 10 litres of air per minute. Tests were then carried out by sampling through two absorbers in series and observing the ratio of isocyanate found in the first absorber to that found in the second, by measuring the optical densities at 544 mμ with a Unicam SP600 spectrophotometer. Initial tests were carried out by using dilute hydrochloric acid without added *N*,*N*-dimethylformamide as the absorber solution; the results were—

Amount of tolylene di-isocyanate found in 2 absorbers, p.p.m. v/v	0·040	0·040	0·035	0·035	0·035	0·025
Amount found in 1st absorber, as percentage of total ..	66	66	67	69	69	63

In later tests an absorber solution containing *N*,*N*-dimethylformamide was used; the results of these tests were—

Amount of tolylene di-isocyanate found in 2 absorbers, p.p.m. v/v	0·100	0·055	0·045	0·035	0·025	0·025
Amount found in 1st absorber, as percentage of total ..	93	80	81	90	87	95

Conclusions

The sensitivity of the test has been increased five-fold by reducing the volume of absorber solution used from 15 ml[2] to 3 ml. To ensure the absorption of a sufficiently high proportion of the total isocyanate in only 3 ml of liquid, an absorber giving a high ratio of depth to volume has been designed. The retaining power of the liquid was increased by incorporating 25 per cent. of *N*,*N*-dimethylformamide in the absorber solution, resulting in the retention in the first absorber of 88 ± 8 per cent. of the total amount of isocyanate trapped from a dry nitrogen stream. As this was intended as a rapid field method the accuracy and precision of these results was considered to be adequate.

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The Determination of Small Amounts of Sulphur in Toluene by Reduction with Raney Nickel

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GRANATELLI'S method,[1] in which sulphur compounds are reduced to sulphides by Raney nickel and the hydrogen sulphide produced on acidification is determined, is potentially attractive for the determination of total sulphur in certain types of material, particularly in highly refined hydrocarbons containing extremely small amounts of sulphur. However, when the method was tried in this laboratory on toluene solutions of known sulphur content the recoveries varied between 70 and 85 per cent. of theory.

Modifications in the mode of preparation and use of the Raney nickel catalyst resulted in an increase in the percentage recovery from elementary sulphur, carbon disulphide and 2- and 3-methylthiophen to 95 ± 5 per cent. at a level of 1 to 10 p.p.m. of sulphur.

The modifications deal with the temperature at which the Raney nickel is activated, the detailed instructions for washing the activated catalyst and the immediate use of the catalyst after its preparation.

METHOD

REAGENT—

Nickel - aluminium alloy—50 per cent. of nickel, 50 per cent. of aluminium, for preparing catalytic nickel. Obtainable from British Drug Houses Ltd.

ACTIVATION OF NICKEL CATALYST—

Assemble an apparatus similar in dimensions to that described by Granatelli,[1] and grease the joints lightly with silicone grease. Remove the reduction flask, measure into it 10 ml of 2·5 M sodium hydroxide solution, and raise the temperature to 75° to 80° C. Add, in one portion, 0·5 ± 0·05 g of nickel - aluminium alloy; a vigorous reaction ensues, and precautions should be taken against the mist of caustic liquor ejected from the flask. Set aside the flask for 10 minutes. Decant the supernatant liquid as completely as possible from the flask, and wash down the neck of the flask with 10 to 15 ml of water from (for convenience) a polythene wash bottle. Swirl the water with moderate vigour to disturb the nickel residue sligntly, but avoid entrainment of air. With the minimum delay required for the residue to settle, decant off the water as completely as possible. Repeat the washing of the neck of the flask and the nickel residue twice with water, and then with 10 ml of isopropanol. Decant off the isopropanol, and add a further 10 ml of isopropanol to the flask. Without delay transfer by pipette 10 or 25 ml of a sample expected to contain 1 to 10 p.p.m. of total sulphur, and replace the flask in the apparatus.

PROCEDURE—

Pass nitrogen through the sample solution at a slow rate (about 2 bubbles per second) for 10 minutes before heating is commenced, and then continue as described by Granatelli.[1] The absorber may be connected to the apparatus at the start of the procedure; this de-aerates the contents of the absorber without the necessity of manipulating the gas flow and absorber later in the procedure.

DISCUSSION OF THE METHOD

Treatment of the nickel - aluminium alloy with hot sodium hydroxide solution and the use of freshly prepared catalyst give improved efficiency of reduction of sulphur compounds in general. Certain compounds are affected by the alkalinity of the catalyst; if, for example, the nickel is washed just once with water, only about 20 per cent. recovery of sulphur is obtained from 2- or 3-methylthiophen. However, for the complete reduction of phenylvinylsulphone it is necessary to limit the number of washings with water to one. Elementary sulphur is completely reduced, irrespective of variations in the washing of the nickel.

Limited experiments have shown that a short reduction with triple washed Raney nickel, with subsequent addition of a small amount of sodium hydroxide solution and a further period of heating will decompose mixtures of compounds requiring different alkalinities for their reduction; this may serve as a satisfactory arbitrary procedure that will eliminate the need for knowing what sulphur compounds are present in a new type of sample.

The modified method is eminently suitable for the routine determination of total sulphur in toluene in the 1 to 10 p.p.m. range and should also be applicable to other aromatic and aliphatic hydrocarbons and to alcohols. The process is simple, quiet and relatively free from hazard, and is such that a routine operator can supervise two sets of apparatus at the same time.

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The Determination of Phosphorus in Different Leathers by Oxygen-flask Combustion

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A HISTORY of the development and uses of the oxygen-flask combustion technique of analysis is presented in the review article by Schöniger.[1] This type of analysis is popular because of its simplicity, ease of operation and the comparatively short time involved in making the determination. It is advisable, however, to observe all safety precautions recommended for combustions of this type. Combustions should be conducted behind a safety glass shield with protective covering for the hands. All combustions in connection with this work were noted to be safe and without incident.

The primary objective of this work was to find a suitable rapid method for determining phosphorus, applicable to the analysis of leather. Initial work showed that when nitric acid was used as the absorbent in the combustion flask, as proposed by Fleischer *et al.*[2] or by Barney, Bergmann and Tuskan,[3] the burning sample produced an objectionable amount of sparking with consequent incomplete combustion and loss of sample. Also, the presence of nitric acid caused erratic colour development with the Association of Official Agricultural Chemists (AOAC) colour reagents.[4] The absorbents suggested by Kirsten and Carlsson[5] were found effectively to suppress spark formation during combustion so that samples were rarely lost by sparking.

The combustion chamber consisted of a regular 500-ml short-necked flat-bottomed flask having a 24/40 ground-glass joint with glass stopper. A hole was drilled in the end of the glass stopper to anchor the wire supporting the sample carrier. Wires extending from the glass stopper and the sample carrier were inter-connected so that they could be disengaged by rotation of the stoppered flask. The sample carrier could thus be completely immersed in the absorbent solution for more efficient dissolution of combustion residues.

Flasks were prepared for the combustion by placing in them 5 ml of 0·8 N sodium hydroxide and flushing with oxygen for about 3 minutes. Seven millilitres of saturated bromine water were then quickly added to the contents of the flask from a pre-calibrated transfer pipette having the tip removed for fast delivery. The fuse of the sample was then ignited and the sample assembly plunged into the flask to ensure a closed seal during and after combustion. After combustion, the sample carrier was demounted into the absorbent and the stoppered flask held for 30 minutes with intermittent shaking. The stopper with its wire stem was then washed and removed. Five millilitres of 22 N sulphuric acid were next added, and the contents of the flask with the sample carrier were boiled for 4 minutes over a gas flame. Boiling provided supplementary oxidation conditions, complete bromine removal, and conversion of phosphate complexes to orthophosphates suitable for analysis by the selected colour reagents.

Dependent on the expected phosphorus content in the sample, the entire contents of the combustion flask were transferred quantitatively into a 50-, 100- or 250-ml calibrated flask and made up to the mark. Portions (2, 5 or 10 ml) considered most likely to give full colour development with the colour reagents were placed in separate 25-ml calibrated flasks. Two millilitres of each of the three AOAC colour producing reagents[4] were added to each portion, and the contents of the flasks were then made up to the mark. It was found that 25-ml burettes were satisfactory for adding the reagents, with advantages in speed and convenience over the use of pipettes. However, the AOAC colour reagents are sensitive to high concentrations of acid, and the acidity should be reduced to 21 milli-equivalents or less per 100 ml before the AOAC colour reagents for full colour development are added. Because of the small amounts of phosphorus in the combustion residues of the last three leather samples analysed (see Table I) the acidity was reduced in the combustion flask, the contents were transferred to a 100-ml calibrated flask, 3 ml of each AOAC colour reagent added, and the contents of the flask were made to the mark for colour development. Smaller amounts of colour reagents were found satisfactory for these low phosphorus values and thus a greater volume of water could be used for rinsing and added during transfer of the sample to the calibrated flask.

Readings of the colour intensity developed were made on a Beckman DB spectrophotometer at 650 mμ against a blank solution treated in the same manner as the sample solution. A standard 1-cm glass cell was used, but the cell was sealed and equipped with conduits to allow it to be operated by a GME Automatic Transferator. Satisfactory conduits were made of intramedic polythene tubing of internal diameter 0·055 inch, external diameter 0·075 inch. About 4·6 ml of sample were found to be a convenient amount for transfer. Flushing between samples was not necessary except when there were wide differences in colour intensities.

It was found convenient and desirable to allow the AOAC reagents to react with the sample for a period of about 2 hours to develop full colour and minimise slight differences in reading times. All readings were compared with a phosphate standard treated under the same conditions as the sample portions, *i.e.*, temperature, size of vessel, milli-equivalents of acid present, reaction time, etc.

Table I lists phosphorus values for three reference standards, *viz.*, Syntropan (amprotropine phosphate; 3-diethylamino-2,2-dimethylpropyl tropate phosphate) triphenylphosphine and triphenyl phosphate, a commercial grade dioctadecyl phosphite and five different sheepskin leathers. These leathers were tanned with a phosphorus-containing compound, tetrakis(hydroxymethyl)-phosphonium chloride, in combination with resorcinol, by Windus, Filachione and Happich's

TABLE I

PHOSPHORUS DETERMINED IN CHEMICALS AND LEATHERS BY THE CARIUS AND PROPOSED METHODS

	Weights of sample used in proposed method,	Phosphorus content		
			Found by—	
		Theoretical,	Carius method,	proposed method,*
Chemicals—	mg	%	%	%
Syntropan	4·38 to 7·00	7·64	7·59	7·65
Triphenylphosphine	3·96 to 5·49	11·83	11·78	11·85
Triphenyl phosphate	6·01 to 6·22	9·51	9·28	9·69
Dioctadecyl phosphite, commercial grade	7·85 to 12·21	5·28	4·63	4·65
Origin of leather—				
United States sheepskin	10·40 to 12·00	—	2·68	2·71
Iran sheepskin	10·30 to 32·30	—	2·15	2·16
New Zealand sheepskin	23·52 to 25·41	—	0·40	0·46
Iran sheepskin	27·92 to 29·70	—	0·48	0·48
Iran sheepskin	26·46 to 32·73	—	0·42	0·47

* Standard deviation of the method was 0·025.

method.[6] Phosphorus values obtained by the Standard Carius method[7] correlate with the proposed method. The results obtained on the test substances illustrate the reliability of the proposed method for determining phosphorus content, although the phosphorus is bound in various different molecular combinations.

I thank Miss Oksana Panasiuk for the Carius analyses and Dr. Clyde L. Ogg for his constant interest and helpful advice.

REFERENCES

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6. Windus, W., Filachione, E. M., and Happich, W. F., U.S. Patent No. 2,992,879, July 18th, 1961.
7. Association of Official Agricultural Chemists, *op. cit.*, Section 38.012, p. 644.

Received *January 7th*, 1963

Inexpensive Sampling Device for Gas Chomatography

BY T. DORAN* AND A. D. SPERRIN†

(*Sondes Place Research Laboratories Ltd., Dorking, Surrey*)

INVESTIGATIONS in progress in this laboratory necessitated the use of a gas-sampling device suitable for "on stream" sampling. Commercially available valves were relatively expensive and the delivery dates quoted were unsatisfactory, so the possibility of constructing a suitable valve was investigated.

A simple method has been described[1] in which were used three glass stop-cocks requiring simultaneous operation for optimum performance. It was realised that a multiple valve performing these basic operations should be available commercially. Drallim Industries Ltd. (Bourne Works, Station Approach, Whyteleaf, Surrey) were consulted, and a valve to our specification was quickly supplied from their range of "custom built" valves. It was ready for use and required only the addition of a calibrated sampling loop. The valve was completely satisfactory and so the temporary sampling loop was replaced by a turret device that permitted the size of sample to be changed readily.

Minimum sample volume was limited by the internal volume of the valve (4·7 ml), and the turret volumes were arranged to deliver samples of, nominally, 5, 10, 15 and 20 ml. For convenience, these volumes were achieved by using twin bores connected by grooves and sealed by a rubber gasket secured by the top cap; the lower end of the bores were sealed with neoprene "O" rings. This simple adaptation, made in our workshops, is shown in Fig. 1. The valve was perfect in operation, requiring a simple turn through 90° to effect sample injection.

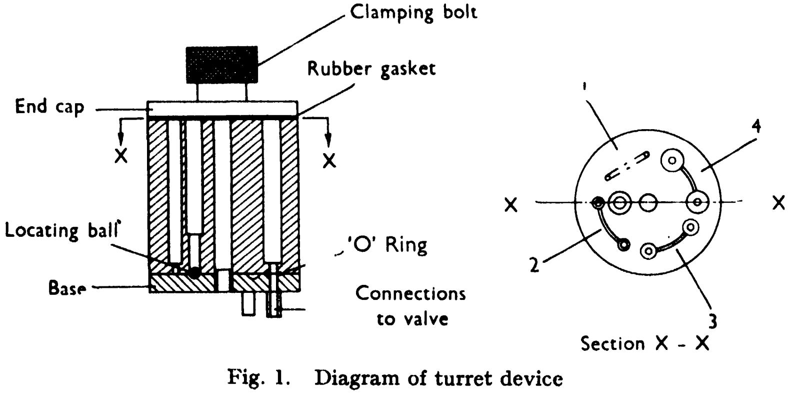

Fig. 1. Diagram of turret device

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*Present address: Energy Conversion Ltd., Chertsey Road, Sunbury-on-Thames, Surrey.

†Present address: Messrs. Nash & Thompson, Instrument Makers, Hook Rise, Tolworth, Surrey.

To extend the range of sample sizes obtainable, various modifications to the basic valve were suggested. In collaboration with Drallim Industries Ltd. a modified valve was produced (supplied at £17 10s.). Although this was more costly than the standard valve (£9 5s.) it was still approximately half the cost of other commercially available sampling valves and had a similar range of application. The turret was made in our own workshop and can be produced cheaply with the minimum of engineering facilities. However, the turret is not essential, and either the standard or the improved valve can be used with fixed sampling loops, as is common with other commercial sampling valves.

The volume of the modified valve is only 1·74 ml, and samples of as small as 2 ml can be dispensed.

TABLE I

TYPICAL RESULTS OBTAINED WITH THE SAMPLING VALVE

Volume of sample dispensed, ml	Integrator counts	Mean	Standard deviation	Coefficient of variation, %
5	365, 373, 367, 368, 365, 366, 367, 368, 366, 366	367	0·8	0·25
10	828, 824, 818, 828, 826, 824, 826, 824, 824, 824	824	0·4	0·05
15	1172, 1170, 1172, 1166, 1168, 1164, 1164, 1166, 1164, 1166	1168	0·6	0·05
20	1562, 1556, 1552, 1554, 1552, 1552, 1550, 1552, 1550, 1550	1554	0·8	0·05

Some typical results obtained when the sampling valve was used are shown in Table I. The counts were obtained by Nogare, Bennett and Harden's method[2] of integration; the deviations noted were probably due to the method of integration rather than to a fault in the sampling valve.

The manufacturers claim the valve to be leak-free to 200 lb per sq. inch, and the results of our experiments show no indication to the contrary. The steadiness of the base-line and the reproducibility of results suggest that leaks, if any, are extremely small. Our investigations involve measurements of down to 1 part of nitrogen in 20,000 parts of hydrogen, and any leak to atmosphere would be immediately visible.

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2. Nogare, S. D., Bennett, C. E., and Harden, J. C., "Gas Chromatography," Academic Press Inc., New York and London, 1958.

Received *February 6th*, 1963

Book Reviews

ANALYTICAL MICROBIOLOGY. Edited by FREDERICK KAVANAGH. Pp. xvi + 707. New York and London: Academic Press Inc. 1963. Price 157s.

Analytical microbiology has been defined as "that branch of microbiology in which micro-organisms are used as reagents for the quantitative determination of certain chemical compounds." As an analytical tool it has proved invaluable for vitamins, amino acids and antibiotics, many of which cannot be measured by purely chemical techniques. This book is probably the most comprehensive so far produced on the subject.

It is no ordinary laboratory manual of methods, but rather a treatise dealing fully with the fundamental aspects as well as describing many assays in detail. The longest chapter is that on antibiotics (157 pages) in which Dr. Kavanagh and eight collaborating authors have given a general survey of the field and described in detail the assay of twenty antibacterial and antifungal substances. The examples have been chosen to illustrate the principles of assaying and to indicate current practices; none of the methods described is necessarily the "best," but all, it is stated, are usable and no claim is made to have produced a compendium of recommended methods for all types of sample.

Vitamins also form the subject of a long chapter (154 pages), each section of which has been written by a specialist. The assays of biotin, folic acid, pantothenic acid and thiamine have received special attention; a section deals with methods for vitamin B_{12} and its congeners in which four different micro-organisms are used, and another describes the *Lactobacillus leichmanii* assay in detail. There is also a section on agar-plate assays with particular reference to pantothenic acid, inositol and pyridoxine.

British workers will be pleased to see the prominence given to large-plate methods, to which a whole chapter by J. S. Simpson has been devoted. As well as dealing fully with the general and statistical aspects of large-plate methods, their application to penicillin and riboflavin is discussed in detail. As Dr. Kavanagh himself points out, the Petri-plate method is inherently inefficient and the greater precision of the large-plate method makes it worthy of more consideration than it has hitherto received in the United States.

The theory of antibiotic inhibition zones is ably discussed by K. E. Cooper in a lengthy chapter. The apparent simplicity of these diffusion tests is deceptive and Dr. Cooper's mathematical treatment should have a salutary effect on those who still rely on absolute size of inhibition zones as sole indications of strain sensitivity.

In his chapter on elements of photometric assays Dr. Kavanagh discusses such questions as the physical factors affecting turbidity and the calibration of photometers. He also examines the importance of such factors as inoculum preparation, incubation temperature and the design and validity of assays. There is an interesting chapter by T. A. Haney, J. R. Gerke and J. F. Pagano on automation of microbiological assays; it will surprise many to learn how far some laboratories have proceeded in this direction.

Finally, mention must be made of G. D. Shockman's chapter on amino acids, which is probably the most extensive report on the subject for many years. In just over one hundred pages he has collected together much of the published work in this field during the last two decades and added a great deal based on his own experience.

For their success, microbiological assays depend on a better understanding of the processes involved and on meticulous attention to details of technique. This book should help from both these points of view and will surely be welcomed in any laboratory specialising in these methods. Its price, however, even by present day standards, is appallingly high, and it is to be hoped that this will not make it inaccessible to those—and they are many—who would enjoy and benefit from reading it.

S. A. PRICE

THE RADIOCHEMICAL MANUAL. Part 2. RADIOACTIVE CHEMICALS. Pp. vi + 78. Amersham, Bucks.; The Radiochemical Centre. (Also available from Her Majesty's Stationery Office.) 1963. Price 25s.

Part 1 of this manual dealt with physical data, presented largely in tabular form (see *Analyst*, 1962, **87**, 916). Part 2 is mainly in text form and deals with chemical aspects of radioisotopes and labelled compounds. Further parts of the manual are stated to be in preparation.

Part 2 begins with a section on sources of radioactive chemicals (21 pages), comprising four chapters on radiochemical manufacture, choice of target materials, separation of primary isotopes, and "a general approach to labelled compounds." A further section on characteristic features of radioactive chemicals (15 pages) consists of three chapters on the purity and instability of radioactive chemicals and on the behaviour of minute amounts and very dilute solutions. The third section (17 pages) has separate short chapters on labelled compounds of carbon, hydrogen, sulphur, phosphorus, iodine and chlorine. Definitions of terms used, 13 Tables, showing synthetic routes to labelled compounds of ^{14}C, ^{35}S, ^{32}P and ^{36}Cl, and an index complete the book.

Most of this book, therefore, provides information on the distinctive properties of radioactive chemicals and on the problems and limitations associated with their use. In addition, in the first section, the reader is quite properly acquainted with some of the problems facing the supplier of radioactive chemicals, *e.g.*, the need for ensuring radiochemical as well as chemical purity, and the stock-holding difficulties created by a variety of factors, such as short half-lives and decomposition by self-irradiation. The information provided must be considered authoritative, in view of the vast experience of the Radiochemical Centre in the preparation and handling of radioactive chemicals. As will be apparent from the length, the book aims to be a guide, rather than a textbook, and references to more detailed information are given at the end of each chapter.

The book is attractively produced and well worth buying.

H. J. CLULEY

SOME GENERAL PROBLEMS OF PAPER CHROMATOGRAPHY: RELATIONS BEWEEN PAPER CHROMATOGRAPHIC BEHAVIOUR AND CHEMICAL STRUCTURE: ATTEMPTS AT SYSTEMATIC ANALYSIS. Edited by I. M. HAIS and K. MACEK. Pp. 220. Prague: Publishing House of the Czechoslovak Academy of Sciences. 1962. Price Kcs. 22.50.

The book is a collection of 32 lectures (of which 3 are only summaries) given by well established authorities in the field of paper chromatography. These and the appropriate discussions have been excellently translated and are presented in a logical order as opposed to their chronological order; this has greatly enhanced the readability of the book.

The book, like the Symposium of which it is a record, is divided into the two sections indicated in the title. Each section is a useful account of modern ideas and techniques and covers aspects of use and interest to inorganic and organic analytical chemists as well as to those more interested in the fundamental aspects of chromatography.

Although all papers are of a high standard, the three introductory papers to the symposium and the two sections and their accompanying discussions are of the highest order, and are well worthy—as is the book in general—of a place in the library of any chemist dealing with paper chromatography.

For students of chromatography, this book is a "must," and its price puts it well within the range of all interested chemists.

L. S. BARK

Publications Received

ZONE ELECTROPHORESIS IN BLOCKS AND COLUMNS. By H. BLOEMENDAL. Pp. viii + 219. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 40s.

THE STRUCTURE OF MOLECULES: AN INTRODUCTION TO MOLECULAR SPECTROSCOPY. By GORDON M. BARROW. Pp. xii + 156. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) $4.35.

HOW CHEMICAL REACTIONS OCCUR: AN INTRODUCTION TO CHEMICAL KINETICS AND REACTION MECHANISMS. By EDWARD L. KING. Pp. xii + 148. New York and Amsterdam: W. A. Benjamin Inc.' 1963. Price (paper) $2.15; (cloth) $4.35.

ELEMENTARY CHEMICAL THERMODYNAMICS. By BRUCE MAHAN. Pp. xii + 155. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) £4.35.

THE SHAPE OF CARBON COMPOUNDS: AN INTRODUCTION TO ORGANIC CHEMISTRY. By WERREN HERZ. Pp. xiv + 152. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) $4.35.

TECHNIQUE OF ORGANIC CHEMISTRY. Volume VIII. Part II. INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS. Edited by S. L. FRIESS, E. S. LEWIS and A. WEISSBERGER. Second Edition. Pp. xii + 703–1582 + Index (pp. 21). New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price £11.

ANALYTICAL CHEMISTRY OF THE ACTINIDE ELEMENTS. By ALFRED J. MOSES. Pp. x + 137. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 45s.

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THE CHEMISTRY OF NATURAL PRODUCTS. Special and Introductory Lectures presented at the Second International Symposium on the Chemistry of Natural Products, Prague, Czechoslovakia. Pp. vi + 215. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 60s.

Reprinted from Pure and Applied Chemistry, *Vol.* 6, *No.* 4. *Pp.* 493–717.

A DICTIONARY OF SCIENTIFIC UNITS: INCLUDING DIMENSIONLESS NUMBERS AND SCALES. By H. G. JERRARD, B.Sc., Ph.D., F.Inst.P., and D. B. MCNEILL, T.D., M.Sc., PH.D., F.INST.P. Pp. 197. London: Chapman & Hall. 1963. Price 21s.

MOLECULAR VIB-ROTORS: THE THEORY AND INTERPRETATION OF HIGH RESOLUTION INFRARED SPECTRA. By HARRY C. ALLEN, JUN. and PAUL C. CROSS. Pp. viii + 324. New York and London: John Wiley & Sons, Inc. 1963. Price 105s.

THE QUANTUM THEORY OF MOLECULAR ELECTRONIC STRUCTURE: A lecture-note and reprint volume. By ROBERT G. PARR. Pp. xvi + 510. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $7.65; (cloth) $11.00.

THERMODYNAMICS OF SMALL SYSTEMS. Part I. By TERRELL L. HILL. Pp. xii + 171. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $6.55; (cloth) $9.90.

PHYSICAL INORGANIC CHEMISTRY. By M. J. SIENKO and R. A. PLANE. Pp. x + 166. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $4.35; (cloth) $7.70.

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING with the assistance of ERNEST B. SANDELL. Part II. ANALYTICAL CHEMISTRY OF THE ELEMENTS. Volume VIII. Pp. xxii + 556. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 150s.

KOMPLEXOMETRIE. Part 1. PRINZIPIEN UND GRUNDBESTIMMUNGEN. By Dr. rer nat. RUDOLF PŘIBIL. Second Edition. Pp. 179. Leipzig: Veb Deutscher Verlag für Grundstoffindustrie. 1963. Price DM 13.80.

ANALYTISCHE CHEMIE. Part I. MASSANALYSE. Part II. GEWICHTSANALYSE. By DIPL.-CHEM. HERBERT RODICKER. Second Edition. Pp. 166; 141. Leipzig: Veb Deutscher Verlag für Grundstoffindustrie. 1961; 1962. Price (Part I) DM 7.80; (Part II) DM 7.50.

NUCLEOPHILIC SUBSTITUTION AT A SATURATED CARBON ATOM. By C. A. BUNTON, Ph.D. Pp. x + 172. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 40s.

Volume 1 of a series of Monographs entitled "Reaction Mechanisms in Organic Chemistry" Edited by Prof. E. D. Hughes, F.R.S.

HANDBOOK OF LABORATORY DISTILLATION. By ERICH KRELL. Edited by E. C. LUMB. Translated from the Second German Edition by C. G. VERVER. Pp. x + 561. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 100s.

PRACTICAL MATHEMATICS FOR CHEMISTS. By F. H. C. KELLY, D.Sc., M.Sc., A.Melb.T.C., F.R.A.C.I., A.Aust.I.M.M. Pp. viii + 148. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 20s.

Reports of the Analytical Methods Committee: Reprints

Nitrogen Factors for Beef, and The Semi-micro Determination of Chlorine in Agricultural Technical Organic Chemicals and their Formulations

THE Report prepared by the Meat Products Sub-Committee, "Nitrogen Factors for Beef," reprinted from *The Analyst*, June 1963, 88, 422–423, and the Report prepared by the Chlorine in Organic Compounds Sub-Committee, "The Semi-micro determination of Chlorine in Agricultural Technical Organic Chemicals and their Formulations," reprinted from *The Analyst*, June 1963, 88, 415–421, are now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1. Price to members 1s. 6d. each; to non-members, 2s. 6d. each.

Reports of the Analytical Methods Committee are only available from the Secretary (not through Trade Agents) and remittances, made out to the Society for Analytical Chemistry, MUST accompany orders.

Errata

JULY (1963) ISSUE p. 546 line 2. For "differences" read "difference." p. 547, Fig. 3. The equations "$Y=0{\cdot}272-0{\cdot}935x$" should read "$Y=0{\cdot}272+0{\cdot}935x$."

OCTOBER, 1963 Vol. 88, No. 1051

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATH

We record with regret the death of

Edmund Frankel.

SCOTTISH SECTION

A Joint Meeting of the Scottish Section with the Institute of Chemistry of Ireland was held on Thursday and Friday, September 5th and 6th, 1963, in the Rupert Guinness Hall Dublin. The Company was welcomed by Mr. M. J. Cranley, M.Sc., F.R.I.C., F.I.C.I., President of the Institute of Chemistry of Ireland. The meeting was divided into four sessions, the chairmen being: Dr. A. K. Mills, F.I.C.I.; Mr. A. F. Williams, B.Sc., F.R.I.C.; Mr. J. K. McLellan, M.A., B.Sc., A.R.I.C., Vice-Chairman of the Scottish Section; and Dr. L. Brealey, B.Sc., F.R.I.C.

An apology for absence from Dr. D. C. Garratt, F.R.I.C., President of the Society for Analytical Chemistry, was conveyed to the meeting by Mr. McLellan.

The subject of the meeting was "Modern Aspects of Chromatography" and the following papers were presented and discussed: "Thin-layer Chromatography of Long-chain Amines," by E. S. Lane, B.Sc., Ph.D., F.R.I.C.; "Thin-layer Chromatography and its Applications to Dyestuffs and Plasticisers," by G. R. Jamieson, B.Sc., F.R.I.C.; "Thin-layer Chromatography—Applications to Steroid Chemistry," by P. Oxley, M.A., B.Sc., A.R.I.C.; "Some Applications of Gel Filtration and Ion-exchange Chromatography to the Fractionation of Polysaccharides," by D. M. W. Anderson, B.Sc., Ph.D., F.R.I.C.; "Ion-exchange in the Study of Metal Complexes in Solution," by J. K. Foreman, B.Sc., A.R.I.C.; "Chromatography in the Identification of Plant Products," by D. M. Donnelly, B.Sc., Ph.D., A.R.I.C.; "Some Recent Applications of Paper Chromatography in the Petroleum Industry," by R. B. Delves, A.R.I.C.; "Recent Applications of Gas Chromatography," by B. A. Rose, B.Sc., Ph.D., A.R.C.S., D.I.C., A.R.I.C.; "A Statistical Evaluation of Gas - Liquid Partition Chromatography as a Method of Quantitative Analysis," by C. E Roland Jones and D. Kinsler; "Determination of Nitro Constituents of Explosives by Gas Chromatography," by A. F. Williams, B.Sc., F.R.I.C.; "The Gas-chromatographic Analysis of Beer," by G. A. F. Harrison, B.A., F.R.I.C., F.I.C.I.; "The Chromatographic Behaviour of Some Nitrophenols on Alumina Impregnated Paper," by L. S. Bark, B.Sc., F.R.I.C., and R. J. T. Graham, M.Sc.; "The Chromatography of *p*-Substituted *p*-Hydroxyazobenzenes on Alumina-impregnated Papers," by R. J. T. Graham, M.Sc., and C. W. Stone, M.Sc., Ph.D., A.R.I.C.

Morning coffee, afternoon tea and lunch were provided each day by Messrs. A. Guinness, Son & Co. (Dublin) Ltd., to whose generous hospitality much of the success of the meeting is due.

On the Thursday evening a bus tour to Glendalough was marred by rain, but nevertheless enjoyed by all who went. The informal dinner on the Friday evening was well attended: the Chair was taken by Professor C. L. Wilson.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 3.30 p.m. on Wednesday, September 18th, 1963, in the Chemistry Department, The University, Edgbaston, Birmingham 15. The Chair was taken by the Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The theme of the meeting was "Contributions to Analytical Chemistry by Younger Members of the Profession" and the following papers were presented and discussed: "Developments in Periodate Oxidation of Organic Compounds," by G. Dryhurst; "Some Aspects of Cathode-ray Polarography," by M. L. Richardson, A.R.I.C., A.C.T.; "The Gas-chromatographic Determination of Impurities in Dichloran," by J. R. Ellis; "Stereoisomerism of *vic*-Dioximes," by S. Thompson; "Phase-solubility Analysis," by R. E. King, A.R.I.C.; "The Friedel - Crafts Acylation of Cycloalkenes," by E. J. Rudd, A.R.I.C.; "Reduction Methods for the Determination of Trace Quantities of Sulphate," by D. B. Adams, M.A., B.Sc., A.R.I.C.; "Proposals for Modifications to the British Standards Dealing with the Analysis of Raw Copper," by K. H. Denmead.

MICROCHEMISTRY GROUP

A SUMMER Meeting of the Group was held on Wednesday, Thursday and Friday, July 17th, 18th and 19th, 1963, at the School of Pharmacy, Brunswick Square, London, W.C.1.

The following papers were presented and discussed: "Future Possibilities in Microanalysis," by Dr. A. J. P. Martin, F.R.S.; "Methods of Organic Microanalysis: a Comparative and Critical Study," by Dr. R. Lévy; "Mass Spectrometry and Microanalysis," by A. Quayle, M.Sc., F.R.I.C.; "Infrared and Microchemistry," by D. M. W. Anderson, B.Sc., Ph.D., F.R.I.C; "Some Recent Developments in Functional-group Analysis on the Micro Scale," by Professor S. Veibel; "The Status of Microgram Analysis," by Professor R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F. There were also two discussion meetings. (For a fuller report of this meeting and summaries of the papers, see pages 745 to 750.)

ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL

THE fourth meeting of the Atomic Absorption Spectroscopy Discussion Panel of the Physical Methods Group was held at 6.40 p.m. on Tuesday, September 10th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Panel, Mr. W. T. Elwell, F.R.I.C.

The discussion was initiated by Dr. J. B. Willis.

JOINT A.B.C.M.-S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

A JOINT Committee of the Association of British Chemical Manufacturers and the Society for Analytical Chemistry on Methods for the Analysis of Trade Effluents has been set up under the Chairmanship of Dr. S. G. Burgess. The other members of the Committee are: (representing the Association of British Chemical Manufacturers) Mr. F. G. Broughall, Mr. L. A. Keelan, Mr. J. G. Maltby, Dr. I. S. Wilson; (representing the Society for Analytical Chemistry) Mr. G. E. Eden, Dr. S. H. Jenkins, Mr. J. G. Sherratt and Mr. N. T. Wilkinson.

The Committee has begun work on reviewing "Recommended Methods for the Analysis of Trade Effluents," published in 1958, with the intention of revision of those methods in need of it. Suggestions for revision from any user of the methods would be welcome, and would assist the Committee by widening the experience on which it can draw; they should be sent to Mr. P. W. Shallis, Secretary of the Analytical Methods Committee, 14 Belgrave Square, London, S.W.1.

Microchemistry Group Summer Meeting, 1963

ONE hundred and eight scientific delegates and fourteen guests attended the Microchemistry Group's three-day Summer Meeting, held in London on July 17th, 18th and 19th, 1963. These dates, immediately following the I.U.P.A.C. Congress, were chosen so that delegates from abroad might be encouraged to attend. In fact, it was gratifying to find that most of the twenty-four overseas visitors, representing the United States of America, Holland, Denmark, Italy, Switzerland, Australia, France, Belgium, Spain and Japan, had come specially for the Summer Meeting. Demand for residential accommodation considerably exceeded the fifty places that had been optimistically reserved in Passfield Hall.

The meeting was centred at the School of Pharmacy, by permission of the Dean, Dr. F. Hartley, who cordially welcomed the Group and its visitors at the initial reception. Dr. R. E. Stuckey, Vice-President of the Society, opened the scientific programme and gave the Society's good wishes for its success.

The object of the meeting was to present accounts of current trends in microchemistry by experts in their fields. As well as the six main lectures, summaries of which are given below, there were two discussion meetings, one on general aspects of microanalysis, introduced by Dr. C. W. Ayres and Mr. R. W. Fennell, and one on instrumental methods of elemental determinations, introduced by Dr. W. Simon, Mr. W. R. Nall and Mr. G. Ingram. Both were stimulating and well supported by brisk discussion from participants.

Delegates were given the opportunity to visit a choice from nine microchemical laboratories in the London area. These visits were much appreciated, and thanks are tendered to the institutions for welcoming their visitors.

A very full programme for scientific delegates included a choice of social events, and there was a varied and enjoyable Ladies' Programme.

At the winding-up Dinner, the President of the Society, Dr. D. C. Garratt, paid tribute to the vigour of the Group and the work of the Sub-Committee that had arranged the meeting.

Future Possibilities in Microanalysis

By A. J. P. MARTIN

DR. MARTIN suggested that future developments in analytical chemistry must be directed towards reducing the scale of the operations. The processes of chemistry were seldom influenced by the quantity of material, and 1000 molecules could react chemically with the same efficiency as 10^{24} molecules. Man's ability to observe and measure much smaller quantities was limited by the size of his hands, and the apparatus he could manipulate.

The advantages of working on a much smaller scale included speed and economy, the latter being essential in many fields of research work. In gas chromatography and ionisation methods the analyst already has tools sufficiently sensitive to work with 10^{-9} g of material, but he needed first of all a balance capable of weighing such quantities. The present method of applying 1 μg to a capillary column was to weigh 1 mg and by a divider throw the rest away. There should be no difficulty in constructing a suitable balance employing similar principles to the ones used at present, but it must be much smaller. To do this it was essential to overcome the problem of the size of human hands.

Dr. Martin believed he now saw how this should be tackled. It was necessary to make a micromanipulator to replace the hands. It would be crude in comparison with a real hand, but given sufficient degrees of freedom, and fingers for gripping, should not be too difficult to operate. A mechanical system for movement and a pneumatic system for the sensory part would probably be the easiest to make and the most useful to operate. It was impossible completely to reproduce the senses of the hands, but sufficient could be reproduced to begin making one-tenth and one-hundredth scale apparatus.

It was interesting to compare this concept of a micromanipulator with previous ones. Although man had used the microscope for 300 years, the only tools available for making manipulations under it were "stone-age" implements—a "spear" and a "battle-axe," which had only three or four degrees of freedom. (The proposed manipulator would have many more than that.)

The properties of this small world must be considered. The mass of the machine went down as the cube of the scale of reduction, and the increased acceleration varied as the reduction in size. Wear and corrosion would be the same as for a larger machine, but the quantity of material used was so small that platinum and diamond could be used. Surface tension would be greater and it would be necessary to work in either a completely wet or a completely dry atmosphere. The operator's ability to see would be diminished—at one-tenth he would become very short sighted, at one-hundredth scale almost blind, and at one-thousandth scale would need to use an electron microscope.

If this reduction in our scale of operations could be achieved, it became possible to imagine a micro feeler catching a molecule and "feeling" its composition. Dr. Feynman, of the California Institute of Technology, who had put forward ideas similar to Dr. Martin's own, had suggested that single molecules could be synthesised by pressing the atoms together.

Methods of Organic Microanalysis: a Comparative and Critical Study

By R. LÉVY

DR. LÉVY reviewed and compared various methods for the micro-determination of elements in organic substances, and included some reference to the effects of a wide range of unusual (hetero-) elements.

For the determination of fluorine, in which the fluoride was measured spectrophotometrically by its bleaching action on iron sulphosalicylate, the oxy-hydrogen flame was the only method he recommended for liquids and volatile compounds, but for other samples the oxygen-flask method could be used, with combustion initiated with a hot silica rod. Use of gelatine capsules always led to low results, but packing in filter-paper with about 15 mg of glucose worked well. The flask combustion had proved itself better for polymers such as Teflon. The spectrophotometric method had a limited precision, and taking 5- to 6-mg sample weights was therefore recommended, standard fluoride solutions being treated at the same time and in an identical manner with the unknowns. Results for phosphorus by the phosphovanadomolybdate colorimetric method were excellent if similar precautions were taken.

For the determination of chlorine, bromine and iodine, five methods of decomposition had been compared. The *oxy-hydrogen flame* had few advantages and required the most skill; iodine could be lost as a solid deposit, and hetero-elements gave more trouble than in other methods. The *Pregl-type bead tube* with detachable absorber was much used without platinum or silica filling, but with at least 100 mm of the combustion tube at over 1000° C. With a series of samples, chlorine or bromine could be determined in 25 minutes. Volatile samples had to be heated with care, but others could be heated automatically. *Oxygen-flask combustion* was cheaper and had little that could break down, such as electric heaters. Generally the bead-tube method was best for routine work and the flask combustion for occasional use, but Dr. Lévy's laboratory had uses for both. Phosphorus, boron, zinc, sodium, manganese and cobalt did not interfere in the determination of chlorine by flask combustion, although some of them interfered in the iodine determination. For similar reasons the *peroxide-fusion* and sulphuric - chromic acid methods were still sometimes used. In the former, fluorine, phosphorus, nickel and zirconium did not interfere, manganese and cobalt could be removed by filtration and tin was the only important element that interfered. The *sulphuric - chromic acid method* (Zacherl and Krainick) was the most widely applicable when hetero-elements were present. It took the most time, but was the easiest to carry out.

Chlorine and bromine were invariably determined by potentiometric argentometry to a pre-determined potential and the condition, *e.g.*, absorbing solutions and volumes, were strictly defined. Iodine was oxidised to iodic acid as in Leipert's method, but by the use of bromine in absence of acetic acid after flask combustion. In presence of chlorine, iodine could be removed by boiling after the addition of more peroxide and sulphuric acid. When chlorine was present together with bromine, the method of correction already published was suitable. This was intended to follow a sodium peroxide fusion, and consequently, when it was used after other methods of decomposition, sodium peroxide had to be added to the solution. Addition of sodium hydroxide and sulphuric acid instead did not give the same results.

For the determination of chloride an electrode of 5- to 6-mm diameter silver rod was used; it was polished daily with fine emery and chamois leather by spinning at 2500 r.p.m. The end-point potential was pre-determined with 5- or 10-ml portions of 0·01 N silver nitrate and 0·01 N potassium chloride (the chloride being added to the nitrate), repeated until results were consistent. Subsequently samples were titrated to this potential by adding excess of silver nitrate and back titrating with potassium chloride.

For bromide the cleaned electrode was stabilised in a silver bromide suspension and its stability was checked by repeated titrations of potassium bromide with 0·002 N solution of silver nitrate; here the back titration was not used.

In conclusion, Dr. Lévy considered the effect of many hetero-elements on the carbon and hydrogen determination using either the Zimmermann filling of copper oxide and silver or the Večeřa filling of cobalt oxide on pumice grains at 750° C. The latter method was shown to have certain advantages.

Mass Spectrometry as a Microanalytical Technique

By A. Quayle

Mr. Quayle said that the first working mass spectrograph had been built by Aston in 1919, and this had set the pattern on which succeeding instruments had been based.

The substance under analysis was excited by an electron beam into giving off streams of positively charged particles. These were focused by passing them through a pair of slits; they were then subjected to electrical and magnetic fields, and the resulting rays focused on some form of detector, either a photographic plate or an ion collector connected to a suitable amplifier. He showed that the amount of deflection of the beam by the magnetic field was proportional to the mass and charge of the particles, by the following mathematical treatment.

The fields deflected the charged particles in an arc. Hence a particle experienced a centrifugal force equal to Mv^2/r. This force was caused by the field H, which was exerting a force HeV. Hence—

$$HeV = \frac{Mv^2}{r} \quad \text{or} \quad r = \frac{Mv}{eH} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \qquad (1)$$

But the kinetic energy of the particle after acceleration was the same as its potential energy, *i.e.*—

$$\tfrac{1}{2}Mv^2 = eV \quad \text{or} \quad v = (2eV/M)^{\frac{1}{2}}.$$

Substituting for v in equation (1)—

$$r = \left(\frac{M^2 2eV}{e^2H^2M}\right)^{\frac{1}{2}} = \left(\frac{2MV}{eH^2}\right)^{\frac{1}{2}},$$

which, when rearranged, gave $M/e = r^2H^2/2V$, and if the fields H and V were constant, $(M/e)^{\frac{1}{2}}$ varied as the deflection (as measured by r). Thus in a beam of ionised particles of equal energy the different masses could be distinguished by the variation in deflection.

In practice the magnetic or electric fields were varied so that ions of different masses were brought in turn to the collector. There were various methods of recording this deflection —focusing the ions on a photographic plate, or measuring the electrical discharge, either by means of a galvanometer spot on photographic paper or by other devices. For qualitative work plane plates could be used, but for accurate quantitative work, concave plates were preferable. When the readings were likely to be indistinct, they could be intensified by making a longer exposure.

Gases could readily be handled, and hydrocarbons up to a five-carbon chain had been analysed at room temperature. The size of the sample used was 2 litres at a pressure of 0·1 mm of mercury (approximately 0·3 ml at S.T.P.). Even smaller samples could be used, and analyses had been carried out on 1 cu.mm of gas, *e.g.*, the gas bubbles formed in plate glass during manufacture.

Volatile liquids (having a vapour pressure of more than 30 μ at room temperature) could also be examined, the sample being introduced into the receiver through a tiny orifice. About 1 μl of liquid was required. Samples having a low vapour pressure might have to be heated and so had to be thermally stable.

Solids also had been examined, about 1 mg of sample being introduced into the apparatus, but nine-tenths of the sample was pumped away before analysis.

One method of introduction was to cover a sintered plate with molten gallium and to introduce the sample as a liquid under the metal by a syringe. The sample was then pumped through the sintered plate into the discharge chamber. Thermally unstable or volatile samples could be introduced directly into the chamber through a pipe. Solid samples were introduced into the electron beam as pellets. Samples having molecular weights of up to 800 had been analysed by these techniques.

Mr. Quayle described in detail some of the applications of the technique. He illustrated the analyses of monatomic and diatomic gases by taking fluorine, krypton and nitrogen as examples. Typical spectra such as those given by air and hydrocarbons were discussed in detail, the examples taken being the analyses of methane, ethane, propane and butane. Other materials whose analysis the speaker described were methyl esters, polychloronaphthalenes and alkyl phosphates. The instrument could be used semiquantitatively for the analysis of gas mixtures and was finding increasing application in conjunction with gas chromatography.

Infrared and Microchemistry

By D. M. W. Anderson

Dr. Anderson began by affirming that infrared spectroscopy should be available in every analytical laboratory since it facilitated not only qualitative analysis, but also quantitative analysis and structural determinations on both organic and inorganic molecules. Furthermore, this important modern analytical tool should be of particular interest to microchemists, since analyses could be made on the microgram scale with standard equipment; sub-micro work was possible with the aid of specially designed cells, or auxiliary instrumentation (often comparatively expensive, *e.g.*, beam condensers, scale expansion, microscope attachments), or both, the present useful limit of sensitivity being approximately 10^{-7} g.

After pointing out the extent to which infrared spectroscopy could be used in research, development, and production control in almost every branch of chemical industry, the lecturer indicated that the lecture time would be more profitably spent in considering fundamental principles and experimental techniques, with particular reference to those available for quantitative analysis, rather than discussing an arbitrary selection of recent specialised applications.

Dr. Anderson then outlined very briefly the origins of spectra, interaction of energy with molecules and the requirements for a molecule to absorb in the infrared region. Stressing that modern physical instruments should not be regarded as "black boxes," he described the essential design features of a modern spectrophotometer and discussed the necessity for, and analytical advantages of, double-beam operation. The choice of prism and window materials for operation in the near, normal, and far infrared regions were described, with particular reference to some less common materials and those recently introduced, *e.g.*, "Irtran" glass (four types of which were now available). The analytical advantages of working in the "near" and "far" regions were indicated.

After describing the various techniques for obtaining spectra for solids (organic and inorganic), liquids (aqueous and non-aqueous) and vapours, Dr. Anderson referred to some analytically useful physical applications and then indicated, very briefly, how the qualitative interpretation of a complex spectrum was attempted, stressing that considerable experience was, in general, necessary for success in this type of infrared work.

The remainder of the lecture, which was extensively illustrated with slides, was devoted to a more detailed discussion of methods of quantitative analysis of single components and of complex mixtures. In concluding his lecture, Dr. Anderson drew attention to the recent increased activity in the application of infrared methods to inorganic systems and to aqueous solutions, to the use of polarised radiation, and to the interesting advances that were being made with the aid of the total-attentuated-reflectance technique introduced in 1961.

Some Recent Developments in Functional-group Analysis on the Micro Scale

By Stig Veibel

Professor Veibel stated that micro-elemental analysis permitted the establishment of the empirical, but usually not the structural, formula of an organic compound. For this, functional-group analysis was more important, being the equivalent of the ionic reactions of the elements in inorganic analysis, but it had to be remembered that mutual interference of the different functional groups was much more serious than the mutual influence of the different elements.

Chemical methods had in recent years been supplemented by physical methods such as chromatography (column, paper, gas, thin-layer) and spectroscopy (ultraviolet, infrared, nuclear magnetic resonance, micro-wave, mass), which facilitated the use of micro techniques for the identification of organic compounds.

Three different steps in the identification had to be considered, *viz.*: the ***detection*** of the presence of a particular functional group, the ***characterisation*** of the compound through the preparation of a derivative or through determination of a physical constant (m.p., n_D, etc.) and the ***determination*** of the equivalent weight of the compound with respect to the functional group considered. Commission I of the Division of Analytical Chemistry of I.U.P.A.C. had started work on a Report on Reactions and Reagents useful for these three purposes.

DETECTION OF THE PRESENCE OF A PARTICULAR FUNCTIONAL GROUP—

Feigl's spot tests had been extended to cover many functional groups, but as most of the reactions were colour reactions, they were not sufficiently specific to be fully trusted unless subsequently corroborated by more specific tests. For this the use of reagents such as 2,4-dinitrophenylhydrazine and other hydrazine derivatives, aralkylthiouronium halides, fluoro-2,4-dinitrobenzene, or other classical or new reagents, had been considerably developed in recent years.

CHARACTERISATION OF FUNCTIONAL GROUPS THROUGH DERIVATIVES—

The reagents described above would often permit the preparation of crystalline derivatives under the microscope. Physical constants, such as the melting-point, could then be determined directly under the microscope, but it had to be remembered, *e.g.*, that all hydrazine derivatives of carbonyl compounds were able to exist in *syn* and *anti* forms, which in the neighbourhood of the melting-point easily underwent mutual interconversion, causing a depression of the melting-point. Other derivatives, such as the aralkylthiouronium salts, were easily decomposed at elevated temperatures, and the rate of heating of the bath should therefore be recorded together with the melting-point found.

R_F values (paper or thin-layer chromatography) or retention times (gas chromatography) were, for some derivatives, more useful than melting- or boiling-points. Infrared spectra were also of great value for the characterisation of different compounds containing particular functional groups. Mr. A. Jart had determined the infrared spectra of some 300 benzylthiouronium salts and of some 150 *p*-bromophenyl esters of carboxylic acids. These spectra were to be published in *Acta Polytechnica*.

The latest development was a combination of a gas chromatograph with a mass spectrograph. It was claimed that this method was applicable to samples from 10 g down to 10^{-9} g.

The investigation of the specificity of the various reagents suggested has not always been sufficiently thorough. It is to be hoped that the above-mentioned I.U.P.A.C. report will be critical, especially in this respect.

DETERMINATION OF FUNCTIONAL GROUPS—

Great progress had been made by the introduction of acid - base titrations in non-aqueous solvents, making possible the titration of substances with very weak protolytic activity, *e.g.*, acids with pK_a values up to 10 or 11 and bases with pK_b values up to 11 or 12. The introduction of titration machines, automatically recording the titration curve, had been a great help.

Progress had also been made with redox titrations. Polarography, which until recently had been used mainly in inorganic analysis, had found applications in organic analysis, too, oscillographic polarography being possibly the most useful variant. By a combination of chromatographic and spectrophotometric methods an automatically recorded investigation of, *e.g.*, protein hydrolysates had been made possible. Other instrumental methods included chronopotentiometry, conductometric titrations and electrophoresis.

References to detailed methods could be found in annual or bi-annual progress reports in most of the journals of analytical chemistry and in the "Organic Analysis" series edited by Mitchell, Kolthoff, Proskauer and Weissberger.

The Status of Microgram Analysis

BY R. BELCHER

PROFESSOR BELCHER explained that the submicro procedures developed at the University of Birmingham for the elementary and functional-group analysis of organic compounds used a sample weight of about 30 to 50 μg, but achieved the same order of accuracy as comparable micro techniques. This order of sample weight was chosen because it represented about the lowest level with which it was possible to work without the use of magnification and manipulators. It was not envisaged that these sub-micro procedures would replace existing micro techniques for routine analysis, but rather that they would be employed for those determinations, particularly in biochemistry, where only very small amounts of material were available.

The first method developed had been a sealed-tube digestion for nitrogen-containing compounds, sulphuric acid being used. Ammonia in the digest was eventually oxidised by an excess of hypobromite, the excess being determined iodimetrically. Sealed-tube decomposition with sodium metal was applied to the determination of chlorine, bromine and iodine. The last two elements were conveniently and easily evaluated by amplification titrations (Van der Meulen and Leipert, respectively). Chlorine had proved more troublesome; an indirect amplification procedure based on the use of mercury$^{\text{I}}$ iodate had been developed, but the technique was extremely exacting. Fluorine was determined by oxygen-flask combustion, followed by colorimetric determination of fluoride with alizarin complexone and cerium$^{\text{III}}$. A sub-micro Carius digestion was used to determine sulphur. The barium sulphate formed was filtered, then dissolved in an excess of ammoniacal EDTA and the excess of EDTA back-titrated with magnesium solution with Solochrome black 6B as indicator. Although a successful combustion technique had been developed for carbon, it had not proved possible to determine hydrogen at the same time because of losses of water by absorption on the surface of the silica apparatus. The carbon dioxide formed by the combustion was frozen out and eventually determined manometrically. Some procedures had been developed for functional groups, particularly for alkoxyl, alkalimino and carboxyl groups and some non-aqueous titrations had been investigated.

Considerable attention had been focused on the determination of hydrogen, almost every conceivable approach having been tried, even methods that would involve its determination independently from carbon, *e.g.*, the Feigl spot-test reaction for hydrogen by heating the sample with sulphur and evaluation of the resulting hydrogen sulphide. Recent work had shown promise with a combustion procedure in which the water formed was immediately trapped by a desiccant. The carbon dioxide was determined manometrically; then the desiccant was heated, the evolved water passed over heated carbon, the resulting carbon monoxide subjected to the Schutze reaction, and the carbon dioxide from this determined manometrically.

Several successful procedures had been developed for application after a sub-micro oxygen-flask decomposition of the sample. Chloride was evaluated mercurimetrically in an ethanolic medium with diphenylcarbazone as indicator. Bromide and iodide were determined, as before, by amplification titrations. Sulphur, as sulphate, was titrated with barium perchlorate in an ethanolic medium with Thorin as indicator. Previous studies of the determination of phosphorus (and arsenic) by means of a sealed-tube decomposition with nitric acid had indicated substantial losses of phosphorus on the walls of the tube. This effect was also present in the flask combustion, but it had proved possible to eliminate the difficulty. After flask combustion, phosphate was determined *via* a titrimetric quinoline phosphomolybdate procedure or a molybdenum-blue spectrophotometric finish. For arsenic, only a molybdenum-blue finish had proved possible.

Much work was currently being carried out on functional groups. The nitro group was being determined by reduction with excess of titanium$^{\text{III}}$ sulphate by using a special sub-micro storage - delivery system for the reagent. Excess of iron$^{\text{III}}$ was then added and the excess back-titrated with the titanium$^{\text{III}}$. This procedure had been successfully extended to the determination of the nitroso group, but, as on the micro scale, results had been erratic for azo groups. It had not so far proved possible to differentiate between aldehydic and ketonic carbonyl, but a general procedure for the carbonyl group had been evolved based on an oximation reaction and eventual non-aqueous titration of hydroxylamine. The bromine chloride method was being investigated as a possible general determination of unsaturation. Non-aqueous titration of the thiol group with mercury$^{\text{II}}$ perchlorate had shown promise. Remarkable success had been achieved with the Malaprade reaction—periodate titration of glycol groups. On the sub-micro scale it had proved possible to titrate the formaldehyde, formic acid and excess of periodate in a single solution, whereas three aliquots were normally required. A method for determining molecular weight was based on depression of the freezing-point of camphor.

A Review of the Methods Available for the Detection and Determination of Small Amounts of Cyanide*

By L. S. BARK AND H. G. HIGSON

(Department of Chemistry and Applied Chemistry, The Royal College of Advanced Technology, Salford, Lancashire)

SUMMARY OF CONTENTS

- Introduction
- Non-colorimetric methods
  - Titrimetric methods involving visual end-point detection
  - Titrimetric methods involving instrumental end-point determination
  - Polarographic methods
  - The use of gas chromatography
- Colorimetric methods
  - Methods involving formation of a metal complex
  - Other colorimetric methods
  - Colorimetric methods based on the König reaction
- Conclusion

THE detection and determination of small amounts of cyanide ions are important because of the extreme toxicity of cyanide to living matter. Cyanides are used in electroplating, in precious metal refining, in case hardening of steels and in many other processes; in the gas industry, many atmospheres and effluents contain cyanides, *e.g.*, the effluents from coke ovens and other gas-making plant. Hydrogen cyanide is used extensively as a rapid and conveniently applied fumigant for food-storage plants, such as warehouses and the holds of cargo ships.

Because of the extreme toxicity of cyanide, low maximum concentrations are allowed in water (which may be used for drinking purposes) and in atmospheres (where human beings may be working). The World Health Organisation[1] recommends that water containing more than 0·01 p.p.m. of cyanide (as CN^-) should be rejected as unfit for public use (domestic supply); the toxic limit for fish is 0·03 p.p.m. The maximum allowable concentration in atmospheres in the United States of America[2] is 10 p.p.m. (5 mg of HCN per cubic metre of air).

These low limits mean that it is essential to use extremely sensitive tests for detecting and determining cyanide. For several years there has been a steady increase in the extent and variety of the methods available for detecting and determining cyanides, which are found not only as "free cyanides" (from HCN, KCN and NaCN) and unstable cyano-complexes, such as $[Zn(CN)_4]^{2-}$, but also as relatively stable complex cyanides, such as ferrocyanides and cobalticyanides, which, although not showing typical cyanide properties, are still toxic and classified by most health authorities with cyanides.

Most of the methods used for detecting and determining cyanide involve the formation of hydrogen cyanide. Of the suggested procedures for decomposing complex cyanides to give hydrogen cyanide (either for detection or determination), that proposed by Williams,[3] in which the solution of simple and complex cyanides is distilled after addition of an acid solution of cuprous chloride, is recommended by British authorities[4]; and Kruse and Mellon's method,[5] in which the complex cyanides are decomposed by distillation with phosphoric acid in the presence of citric acid and ethylenediaminetetra-acetic acid (EDTA) under partial vacuum, is used by many chemists in the U.S.A. The latter workers[6] also suggested solvent extraction as an isolation method. Serfass, Freeman, Dodge and Zabban[7] used a reflux method with an air current to transfer hydrogen cyanide from the sample to absorption tubes. Decomposition of complex cyanides is hastened by the action of magnesium chloride, mercuric chloride and sulphuric acid, yielding uniform high recoveries of hydrogen cyanide from simple or complex cyanides; boiling under reflux for 1 hour is generally adequate. Complex cyanides of iron, copper or cobalt generally require longer treatment. Schwapowalenko[8] recommended that the hydrogen cyanide be liberated by heating the test solution in the presence of sodium hydrogen carbonate to prevent any interference from volatile oxidising or reducing compounds.

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*Reprints of this paper will be available shortly. For details please see p. 822.

The methods used for detecting and determining cyanide can be divided into two main sections, *viz.*, non-colorimetric and colorimetric; these can be further sub-divided, *e.g.*—

Non-colorimetric—

(*a*) Titrimetric methods involving visual end-point indicators.
(*b*) Titrimetric methods involving instrumental methods for determining the end-point.
(*c*) Polarographic methods.
(*d*) Gas-chromatographic methods.

Colorimetric methods—

(*i*) Methods involving formation of metal complexes.
(*ii*) Other methods.

Some methods are applicable only to the detection and some only to the determination of cyanide, but most of the available colorimetric methods are used for both.

NON-COLORIMETRIC METHODS FOR DETECTING OR DETERMINING TRACE AMOUNTS OF CYANIDE

The detection of hydrogen cyanide by smell can be one of the most sensitive tests (0·001 p.p.m. can be detected), but many people are relatively insensitive to the smell.

TITRIMETRIC METHODS INVOLVING VISUAL END-POINT DETECTION—

The earliest reported method for determining cyanide is Liebig's titrimetric method,[9] based on the formation of turbidity due to silver cyanide after all the cyanide has reacted with standard silver nitrate solution. An argenticyano-complex is formed initially, and then a slight excess of silver nitrate produces turbidity due to the formation of silver cyanide. This titration is subject to error in alkaline[10,11] and ammoniacal[12,13] solution. A similar method proposed by Denigès[14] is based on the turbidity due to silver iodide in the presence of ammonium hydroxide with potassium iodide as indicator; although this method gives high results in the presence of a large excess of ammonia,[12,13,15,16] accurate results are obtained if the concentration of ammonium hydroxide is carefully regulated.[12,13,15,17] Beerstecher[18] modified the Denigès silver nitrate titration; he used a photo-electric colorimeter or turbidimeter for end-point detection, and delivered 0·001 M silver nitrate solution from a piston microburette. Ryan and Culshaw[19] reported the use of *p*-dimethylaminobenzylidene rhodanine as an indicator in Liebig's method, and this modification was recommended by the American Public Health Association[20] for determining cyanide concentrations of 1 p.p.m. and more. For concentrations greater than 10 to 20 p.p.m., the British recommended method[4] is also the Liebig method, which is therefore still widely used. Ricci[21] published a paper on the inter-relations of the equilibrium constants in aqueous solutions saturated with silver cyanide, and considered mathematically the relation of the point of precipitation of silver cyanide or of silver iodide, as the titration end-point, to the equivalence-point in the argentimetric titration of cyanide. In a second publication[22] he considered the shape of the titration curve, or the graph obtained by plotting a function of the concentration of silver$^{\text{I}}$ ion present (p[Ag$^+$]) against the amount of silver nitrate added, to determine the position of the inflexion-point in the curve and its relation both to the equivalence-point and to the point of first appearance of a precipitate. Two sets of conditions were examined mathematically—

(*i*) the titration of pure aqueous potassium cyanide with silver nitrate, in which the hydrogen ion concentration is variable during the titration, and

(*ii*) the titration of potassium cyanide in presence of excess of ammonia—in this condition the value of the hydrogen ion concentration is practically constant, being fixed mainly by the ammonia.

Under the first conditions the precipitation of silver cyanide would precede the inflexion-point, which would occur only in the supersaturated solution, but the precipitation of silver cyanide, if it should become visible when the solution first becomes theoretically saturated, would give only a negligible (negative) error when taken as the end-point. The inflexion-point occurs before the equivalence-point, but the titration error with the inflexion-point as the end-point would be extremely small. In the presence of excess of ammonia, the inflexion-point occurs at the equivalence-point. Ricci worked out mathematically that the precipitation of silver cyanide (unless the concentration of potassium cyanide was large) occurred after the equivalence-point, leading to a small (positive) error. The precipitation of silver iodide occurred before the inflexion-point. The calculations would suggest that the inflexion-point, without potassium iodide, would be a much better end-point. The importance of this theoretical mathematical treatment is that the best conditions may be selected for titrating different concentrations of cyanide with silver nitrate solution.

Other titrimetric methods involving use of different indicators and titrants have been reported: diphenylcarbazide[23,24] and calcein[25] have been used as indicators in argentimetric titrations; titrations involving the formation of a mercury cyanide have been described by Tanaka and Yamamoto,[26] Gregorowisz and Buhl[27] and Kraljic,[28] who used cupric diethyldithiocarbamate, Variamine Blue and nitrobenzene (with ferrocyanide), respectively, as indicators. It is often necessary before titration to separate the cyanide as hydrogen cyanide by distillation to avoid interference from organic substances and oxidising and reducing agents. Wrónski[29] reported the independent titration of cyanide and sulphide; he used *o*-hydroxymercuribenzoic acid to complex the sulphide and titrated with standard nickel sulphate solution, with murexide as indicator. He also suggested the use of thiofluorescein as an indicator in the argentimetric determination of cyanide; the colour change is, however, not very sharp, and the indicator is not widely used. The use of standard nickel solution in the titration of cyanide was also reported by de Sousa,[30] who determined cyanide in the presence of thiocyanate and chloride by adding a known excess of ammoniacal nickel sulphate solution to the sample and titrating the non-complexed nickel with EDTA, with murexide as indicator.

TITRIMETRIC METHODS INVOLVING INSTRUMENTAL END-POINT DETECTION—

Instrumental methods of analysis are frequently used; the potentiometric titration of cyanide with silver nitrate has been described by Treadwell, Waller and Lauterbach,[31] and Clark.[32] Read and Read[33] suggested bimetallic electrode titration and Gregory and Hughan,[34] using the null-point equivalence potential method proposed by Cavanagh,[35] reported that this method is superior to Liebig's method for determining cyanide in plating solutions.[36] Wick[13] reported that potentiometric titration with mercuric chloride as titrant was an accurate method; Thompson,[17] however, stated that the silver nitrate titration of cyanide to $Ag(CN)_2^-$ was more accurate than the titration with mercuric chloride. Laitinen, Jennings and Parks[37] claimed that the amperometric titration of cyanide with silver nitrate is equal in accuracy and precision to the visual Dénigès method, and is applicable at much higher dilution. Direct amperometry with a rotating silver anode and a stationary platinum cathode has been used by McCloskey,[38] who claims a lower limit of linear response at about 0·01 μg of cyanide in a 7-ml sample and 0·0005 μg of cyanide for a 0·5-μl sample introduced into 10 ml of supporting electrolyte. The electrode response and the sensitivity levels change daily and hence must be frequently checked. A similar system has been operated and patented[39] in this country for electrically determining the concentration of hydrogen cyanide in air (in coal-mines, etc.).

Shinozuka and Stock[40] have described the amperometric titration of low concentrations of cyanide with silver nitrate; they used a rotating platinum electrode and a sodium sulphite medium. A potentiometric method, in which an auxiliary current of 5 to 20 μA per sq. cm is used, has been patented[41] for determining cyanide concentrations in solution (particularly solutions obtained in the refining of gold and silver). Electrometric titrations of cyanide by the dead-stop[42] end-point system with 0·1 N silver nitrate[43] and 0·01 N iodine solution[44] have been reported. Baker and Morrison[45] measured the current from the electrochemical cell Ag/NaOH(0·1 M)/Pt, containing cyanide; the cell operates spontaneously as a measure of one of the reactants in the cell (CN^-)—for 2·6 μg of cyanide, a response current of 17 to 18 μA was reported. The coulometric titrations of cyanide with mercury and silver have been compared by Przybylowicz and Rogers,[46] who showed that mercury was slightly superior to silver for the determination of small amounts of cyanide.

POLAROGRAPHIC METHODS—

Anodic reactions of cyanide at a dropping-mercury electrode have been reported. Jura[47] described the determination of cyanide in flowing alkaline solutions with a shielded dropping-mercury electrode; empirically derived correction factors for the polarographic determination of free cyanide in the presence of sulphide were used by Karchmer and Walker,[48] and Hetman[49] reported a method, depending on a pure reduction wave, in which a base electrolyte consisting of pyridine and potassium nitrate with a mercuric salt was used. The last-named method is uncomplicated and permits the determination of cyanide in concentrations as low as 2 p.p.m. with a comparatively simple polarograph.

THE USE OF GAS CHROMATOGRAPHY—

Gas - liquid chromatography has been used for determining hydrogen cyanide. Woolmington[50] used a method in which water vapour and hydrogen cyanide are separated from oxygen, nitrogen, methane and carbon monoxide by passing the sample (with hydrogen as carrier gas) at 100° to 107° C through a column packed with acid-washed Celite (30 to 60 mesh) impregnated with 20 per cent. w/w of polyoxyethylene glycol (molecular weight 1500). Hydrogen cyanide emerges from the column after 4 to 6 minutes, and its concentration is calculated from peak heights. Schneider and Freund[51] attained sensitivity in their method by a 2000-fold concentration step involving a short column cooled in a mixture of solid carbon dioxide and acetone, and amplification of the thermistor-bridge detector output before recording it on a strip chart.

COLORIMETRIC METHODS

METHODS INVOLVING FORMATION OF A METAL COMPLEX—

Some of these methods, although specific for cyanide and of ample sensitivity, produce unstable colours. The formation of thiocyanate[52,53,54,55] from cyanide (by reaction with ammonium polysulphide) and then ferric ferrithiocyanate (by addition of ferric chloride; producing a blood red colour) is an excellent test for cyanides; however, it is extremely unreliable as a method for determining small amounts of cyanide owing to the instability of the ferric thiocyanate colour. The test is applicable in the presence of sulphide or sulphite; if thiocyanate is originally present, the cyanide must first be isolated before the test is applied (sensitivity, 1 μg of CN^-; concentration limit, 1 in 50,000). Another extremely sensitive test for cyanide depends on the formation of Prussian blue[53,56,57,58,59] ($Fe_4[Fe(CN)_6]_3$). Gettler and Goldbaum[57] found that the sensitivity of the Prussian-blue method could be enhanced by conducting the gas, obtained by aeration of the solution containing hydrogen cyanide (at 90° C), through filter-paper impregnated with ferrous sulphate and dilute alkali (sensitivity, 0·2 μg of CN^-). The cyanide (originally present in the sample) could then be determined by comparing the blue stains produced by immersion of the paper in dilute sulphuric acid with standard stains. Hubach[60] modified this method for detecting cyanides and ferrocyanides in wines. For the detection of cyanide, the aeration of the sample was carried out at room temperature (instead of 90° C). For ferrocyanides, use is made of the fact that hydrogen cyanide is liberated quantitatively from soluble and insoluble ferrocyanides by sulphuric acid in the presence of cuprous chloride at temperatures near 100° C. British authorities[61] recommend Gettler and Goldbaum's method for detecting hydrogen cyanide vapour in air. The copper acetate - benzidine test,[62,63,64] sensitive to 0·25 μg of cyanide (CN^-) in a limit of dilution of 1 in 200,000,[65] is one of the most sensitive and easily applied methods for detecting cyanide. The reaction involves the formation of benzidene blue and is due to the oxidation of benzidine by the removal of $copper^{I}$ from the $copper^{II}$ - $copper^{I}$ system, which raises the oxidation potential of the system and the benzidine is oxidised. It is also probable that copper - benzidine complexes are formed. This test is extremely sensitive for cyanide, provided that oxidising or reducing substances are absent; hence Schwapowalenko's[8] recommendation that hydrogen cyanide be liberated by heating the test solution in the presence of sodium hydrogen carbonate. Cullinane and Chard[66] claimed that a four-fold increase in sensitivity resulted if a substituted benzidine, 2,7-diaminodiphenylene oxide, is used in place of benzidine, but we have not been able to substantiate this claim.

The Weehuizen method,[67,68,69,70] which involves the oxidation of phenolphthalein in alkaline solution to the corresponding red phthalein by $copper^{II}$ ions in the presence of cyanide is a recommended method[4] for determining cyanide. It is an extremely sensitive method, but requires careful control; it is not specific for cyanides because erratic results may be caused by minute amounts of foreign oxidising materials.

A test-tube for detecting hydrogen cyanide in air or other gases has been patented.[71] The tube contains a carrier substance, *e.g.*, silica gel, impregnated with silver chloride, mercurous chloride, lead chloride or a chloride of a metal forming water-insoluble or sparingly soluble cyanides (such as those of copper or palladium), and with an indicator for hydrogen chloride, *e.g.*, bromothymol blue or bromocresol green. The prepared tubes are claimed to be insensitive to oxygen (oxidation) and stable for several years.

The ability of the cyanide ion to form stable complexes and cause de-masking of inner complex-bonded transition metals has also been used by various workers for detecting and determining cyanide. Feigl and Feigl[72] reported a colorimetric procedure in which the de-masking of dimethylglyoxime by the action of cyanide on the palladium dimethylgloximate complex permits nickel ions present to form a red dimethylgloximate. An advantage of this test is that it can be made in alkaline solution, and, in contrast to other methods, does not require the previous liberation and evolution of hydrogen cyanide. Other palladium complexes gave analogous results, and, compared with palladium dimethylgloximate, the palladium salt of 1,2-cyclohexanedione-dioxime[73] gives increased sensitivity. Feigl and Heisig[74] have also reported the detection of 2·5 μg of cyanide by the de-masking of copper from copper^{II} oxinate, which permits aluminium ions present to form the fluorescent aluminium^{III} oxinate.

Brooke[75] selected palladium α-furildioxime as the most sensitive reagent for determining cyanides in "refinery waste water" in the range 0·5 to 3 p.p.m. of cyanide, and Hanker, Gelberg and Witten[76] used the reaction of cyanide with potassium di-(7-iodo-5-sulpho-oxino)palladium^{II} in alkaline solution. The addition of iron^{III} in acid solution produces a blue colour due to the iron complex with an absorption maximum at 650 mμ. These workers also developed a fluorimetric method depending on the de-masking of 8-hydroxy-5-quinolinesulphonic acid by cyanide from the non-fluorescent potassium di-(5-sulpho-oxino)palladium^{II}. The liberated quinoline derivative then co-ordinates with magnesium ions present to form a fluorescent chelate, which is used for measuring the cyanide concentration. It is claimed that 0·02 μg of cyanide per ml of solution can be determined. Hanker, Gamson and Klapper[77] had earlier developed a method involving the formation of a fluorescent compound by reaction between nicotinamide and cyanogen chloride (formed by the reaction of cyanide and chloramine-T). Although it is rapid, the method is not widely used. Musha, Ito, Yamamoto and Inamori[78] examined the inhibiting action of cyanide ions on the chemiluminescence of luminol (3-aminophthalhydrazide), and determined the cyanide concentration by measurement of the induction period. Yamasaki and Ito[79] indirectly determined cyanide by converting it into a stable nickel complex $[Ni(CN)_4]^{2-}$ at pH 9 to 11 (in presence of an ammonium buffer); the excess of nickel was determined colorimetrically with furil α-dioxime in a suspension at pH 4 containing gelatin, the optical density at 480 mμ being measured.

Several methods involving the de-masking effects of mercury^{II} have been used for detecting and determining cyanide. In one method[80] cyanide is used to liberate 2-hydroxyethyldithiocarbamic acid from its mercury^{II} complex; the liberated acid reacts with copper to form a soluble yellow complex having an absorption maximum at 383 mμ. It is claimed that between 0·2 and 4 p.p.m. of cyanide can be rapidly determined by this method. Some ions interfere, but up to a 60-fold excess of thiocyanate can be tolerated. Tanaka and Yamamoto[81] recently developed a method involving the use of mercuric diphenylcarbazide; paper impregnated with this reagent changes from blue-violet to red in the presence of cyanide (0·25 μg per 0·05 ml) in a neutral or weakly basic solution. With gaseous hydrogen cyanide flowing in a thin glass tube containing a strip of paper, the length of the colour change on the strip of paper is proportional to the amount of cyanide. The sample (approximately 1 ml containing less than 10 μg of cyanide) is heated with sodium hydrogen carbonate in a glass tube. The error is less than 5 per cent., and interference from ferrocyanides is avoided by using cadmium nitrate mixed with the sodium hydrogen carbonate.

Ohlweiler and Meditsch[82] used the colour reaction of mercuric ions with *p*-dimethylaminobenzylidene rhodanine as the basis of an indirect absorptiometric method for determining cyanide, based on the masking of the mercury by reaction with cyanide and measurement of the excess of mercury^{II} by reaction with the organic reagent. These workers also used diphenylcarbazone[83] to react with the mercury not masked by reaction with cyanide, and state that greater sensitivity is thus obtained. Both these methods require the use of a Conway microdiffusion apparatus for preliminary concentration of the cyanide and hence require fairly lengthy test times to obtain results. The methods are not applicable below 0·5 p.p.m. without the use of a differential technique. Hoffman[84] described a similar reaction with diphenylcarbazone; many other ions (iodides, sulphides, etc.) commonly present in effluents interfere.

The ready formation of an argenticyanide complex has been used in a de-masking reaction.[85,86] In the first method,[85] paper impregnated with silver dithizonate is treated with a drop of test solution containing cyanide, and the chromatogram is developed with a mixed water - 0·1 N potassium hydroxide (40 + 1) solution. The area of the white spot (on a violet-pink background) is proportional to the concentration of cyanide. This method is rapid and gives reasonably accurate results; its main advantage is that thiocyanate, chloride and bromide do not interfere. The second method[86] is based on the same principle, but the weakening of the colour of the silver dithizonate in carbon tetrachloride solution is taken as a measure of the amount of cyanide present. When copper, zinc and lead are present in the original solution, they are sequestered with EDTA. For samples containing sulphides or free chloride, the solution is treated with lead nitrate, sodium sulphite or sodium thiosulphate before the addition of EDTA.

Schilt[87] described the spectrophotometric determination of cyanide based on the formation and extraction of the neutral dicyano bis-(1,10-phenanthroline) - iron^{II} complex produced by the exchange reaction between the reagent ferroin and cyanide ions. The method is convenient, relatively free from troublesome interferences and applicable to microgram amounts of cyanide ion and concentrations of the order of microgram per ml (p.p.m.).

An indirect colorimetric method for determining cyanide (and sulphide) with thiofluorescein has been reported by Wrónski.[88] An alkaline solution of thiofluorescein, decolorised with silver nitrate, becomes blue on the addition of hydrogen cyanide or hydrogen sulphide. The phenomenon is used to determine hydrogen cyanide (1 to 7 μg) and hydrogen sulphide (0·5 to 2 μg). For simultaneous determination, the optical density is measured, then a drop of formaldehyde is added to discharge the colour caused by hydrogen cyanide, and the optical density due to hydrogen sulphide alone is measured after 10 minutes. Gregorowicz, Buhl and Śliwa[89] determined traces of cyanide in alkali sulphides by precipitation of the sulphide ions with zinc ions, and then steam-distillation of hydrogen cyanide from the diluted solution into 0·01 N sodium hydroxide and reaction of the acidified distillate with Variamine Blue solution in the presence of copper sulphate solution. In a second paper[90] the colour reaction of copper^{II} and Variamine Blue with cyanide (0·03 μg per ml), thiocyanate (40 to 200 μg) and iodide (1 to 10 mg) was reported. In the determination of cyanide, a 100-fold excess of sodium, potassium, ammonium, chloride, nitrate and sulphate ions may be present without any ill-effects.

OTHER COLORIMETRIC METHODS—

The use of picric acid[91 to 98] involves simple procedures, easily prepared reagents and a stable developed colour. Although it is not a very sensitive method it has recently been used[99] for determining the residual cyanide in and on apples after fumigation with hydrogen cyanide.

Alkali cyanides and complex cyanides can be detected to a limit of 0·05 μg of cyanide[100] by their catalysis of the benzoin condensation in alkaline solution. The benzoin formed is readily detected by the violet colour obtained on addition of *o*-dinitrobenzene.

COLORIMETRIC METHODS BASED ON THE KÖNIG REACTION—

For the detection and determination of small amounts of cyanide in trade effluents, the method recommended by the Joint Committee of the Association of British Chemical Manufacturers and the Society for Analytical Chemistry[101] is based on the method developed by Aldridge,[102] which is an example of the König[103] synthesis for pyridine dyestuffs, in which cyanogen bromide or chloride reacts with pyridine and an aromatic amine to form a dyestuff. Aldridge's method consists in allowing the cyanide to react with excess of bromine, removing the excess of bromine with arsenious acid solution, and then allowing the cyanogen bromide formed to react with a mixed pyridine - benzidine reagent. An intense orange colour is formed, which changes to red during 6 minutes, according to Aldridge, and is stable for 6 to 24 minutes at 20° C when measured with a Spekker spectrophotometer in 1-cm cells and a 2·303 Ilford blue filter. In his second paper improvements are described, and, by using an Ilford 604 green filter, he claims that an increase in sensitivity of 75 per cent. is obtained with a colour stability of 30 minutes; 0·2 μg of hydrogen cyanide at a concentration of 0·1 μg per ml could be determined with an error of ±2 per cent. by this modified method. Krawczyk[104] reported that the method gives a perceptible orange colour with as little as 0·1 μg of hydrogen cyanide per ml on 100 ml of sample. In the method, cyanide and thiocyanate are determined together, and the thiocyanate is then determined in a separate portion of the acidified and aerated sample; the cyanide is obtained by difference.

Bruce, Howard and Hanzal[105] modified Aldridge's method to determine cyanide and thiocyanate separately, rather than the cyanide by difference. They used three aeration tubes in series; A (air-washing tube) contained 20 per cent. sodium hydroxide solution, B contained 20 per cent. trichloroacetic acid solution and C contained 0·1 N sodium hydroxide solution. The sample containing cyanide and thiocyanate ions was placed by pipette in tube B, and aeration was begun. After 15 minutes' aeration, the cyanide collected in tube C was determined by Aldridge's method. The thiocyanate left in tube B was also determined after aeration.

Saltzman[106] used Aldridge's original procedure and reported that the final colour was unstable, changing in both tint and optical density; the instability of the colour increased with temperature. Colour measurement was made at 510 mμ. Good results were obtained by controlling the temperature and by allowing for the age of the sample colour by means of a set of readings made at various ages of the standards used.

Whereas Aldridge used filters (initially 2·303 Ilford blue, then Ilford 604 green) that transmit light over a relatively broad spectrum region, other workers have used a definite wavelength. These wavelengths have differed rather widely—480, 510, 520 and 532 mμ, which, to some extent, is an indication of the many divergent view-points about this method.

For coloured or turbid samples or samples containing low concentrations of cyanide, Aldridge's method has been modified by Nusbaum and Skupeko[107]; the determination is carried out in the presence of n-butanol, the dye formed is thus extracted with the n-butanol and the sensitivity of the method is extended (0·02 to 0·5 p.p.m.). For samples containing a large excess of sulphide ions, Aldridge's procedure was modified by Baker *et al.* [108] The sulphide ions are oxidised to sulphuric acid with bromine at the same time as cyanide ions are brominated to form cyanogen bromide. This avoids the necessity for prior removal of the sulphide ions from the sample. The method is sensitive to less than 0·5 μg of cyanide in the presence of 2500 μg of sulphide (error ±5 per cent. at a concentration of 10 μg of cyanide).

Russell and Wilkinson[109] have modified Aldridge's method to make possible the determination of cyanide in the presence of thiocyanate, ferrocyanide and ferricyanide, and also in colour solutions. The complex cyanides are "fixed" with zinc acetate solution and the cyanide (as hydrogen cyanide) is distilled from the acidified solution into 0·1 N sodium hydroxide and then determined with a pyridine - benzidine reagent after formation of cyanogen bromide. (The pyridine - benzidine reagent is not the same as that recommended by Aldridge.) Up to 3 p.p.m. of CN^-, present as cyanide ions, can be measured in the presence of up to 25 p.p.m. of CN^-, present as thiocyanate, and up to 5 p.p.m. of CN^-, present as complex ferro- and ferricyanides, in aqueous solutions containing all these substances and also in the presence of sodium and calcium chlorides. The method is accurate to within 0·05 p.p.m. of CN^- at the maximum concentrations of these substances and to within 0·01 p.p.m. of CN^- at lower concentrations. Aldridge's method has been used for a wide range of materials, including the determination of cyanide in biological materials[110] and foodstuffs.[111,112] For biological materials a preliminary distillation step is carried out. For foodstuffs, a modified Conway cell is used to effect a preliminary separation of the cyanide by microdiffusion.

Murty and Viswanathan[113] used barbituric acid in place of benzidine. They used 25 ml of sample (0·01 to 1·5 μg of cyanide per ml), formed cyanogen bromide and incubated the solution at 40° C for 40 minutes to allow colour formation after addition of a mixed pyridine - barbituric acid reagent. This method was claimed to be more satisfactory, since the colour is stable for approximately 3 hours. Kratochvíl[114] oxidised cyanide to cyanogen chloride with chloramine-T solution, and allowed the cyanogen chloride to react with pyridine to form glutaconaldehyde, which reacts with dimedone to produce a violet colour that can be measured, after 30 to 40 minutes, at 580 to 585 mμ. Desmukh and Tatwawadi[115] made use of the fact that cyanides and thiocyanates develop a yellow colour when treated with chloramine-T solution in the presence of pyridine. Schulek, Burger and Fehér[116] reported a method for determining ammonia, cyanide, nitrite and nitrate when present together.

Ammonia and cyanide are distilled off, ammonia is determined by titration of the distillate and the cyanide is then determined by the cyanogen bromide - iodide reaction.

A method similar to Aldridge's method and also based on the König reaction is that proposed by Epstein.[117] Cyanide is oxidised to cyanogen chloride with chloramine-T, the cyanogen chloride is then allowed to react with pyridine containing 3-methyl-1-phenyl-5-pyrazolone and a small amount of bis-(3-methyl-1-phenyl-5-pyrazolone), and the blue colour produced is measured at 630 mμ. The excess of chloramine-T is reduced by the pyrazolone, but since this will also reduce the cyanogen chloride, the pyridine must be added to avoid this. If the "bis-pyrazolone" is not used, a blue colour is still developed, but it is not stable. Epstein's method is sensitive and may be used in acid, neutral or slightly alkaline media; it is recommended by the American Public Health Association.[20] It has been used for determining cyanide in fish tissue[118] and in wines treated with ferrocyanide.[119] Dodge and Zabban[120] and Balla and Bene[44] also described the use of Epstein's method for determining cyanide and a copper - pyridine reagent for thiocyanate. The cyanide was separated from thiocyanate by extraction as hydrogen cyanide from acidic (glacial acetic acid) solution with four 40-ml portions of isopropyl ether. The hydrogen cyanide was re-extracted from the isopropyl ether with three 30-ml portions of 3 per cent. sodium hydroxide solution. The extraction was reported to be over 95 per cent. efficient.

Ludzack, Moore and Ruchhoft[121] considered that three published methods offered the most promise for the determination of cyanide in water and waste samples: (*a*) Aldridge's method as modified by Nusbaum and Skupeko[107]; (*b*) Epstein's method; (*c*) the modified Liebig titration with the use of Ryan and Culshaw's *p*-dimethylaminobenzylidene rhodanine indicator.[19] They concluded that all samples should be treated by acid distillation except when experience had shown that no difference occurs in the results obtained with or without distillation. Certain samples containing interfering substances that could be steam distilled may require solvent extraction before analysis can be attempted. When this is so, 2,2,4-trimethylpentane (iso-octane), hexane or chloroform will remove the soluble interfering substances without extracting more than traces of cyanide. Epstein's method was preferable for cyanide below 1 p.p.m. (Sensitivity could be extended to 5 parts in 10^9 by use of a colour extraction procedure with n-butanol.) When more than 1 p.p.m. of cyanide was to be determined, the silver nitrate titration was recommended.

Other workers who favour Epstein's method are Whiston and Cherry.[122] They discontinued using Aldridge's method owing to the carcinogenic properties of benzidine, and obtained a straight-line calibration graph over the range 0 to 2 p.p.m. with Epstein's method.

CONCLUSION

From the review of methods available for determining small amounts of cyanide ions it is apparent that colorimetric methods are the best for amounts such as 0·1 to 1·0 p.p.m. Sensitivity in this range is evident in the methods involving use of phenolphthalein (or *o*-cresolphthalein) or Prussian blue, or the methods based on the König synthesis, namely Aldridge's method and Epstein's method. Of these methods the phenolphthalein and *o*-cresolphthalein methods are non-specific for cyanides and must usually be applied to samples that have been concentrated by distillation. The Prussian blue method requires the volatilisation of the cyanide and its absorption on treated filter-paper; it is thus subject to similar errors of separation. Small amounts of ferro- or ferricyanides, if present in the sample, tend to break down on distillation, releasing cyanide and causing appreciable errors in the micro-determination of cyanide in the original sample. The two methods based on the König synthesis permit the determination of cyanide directly on the original sample. These two methods are generally considered to be the best for small amounts of cyanide, and in fact both have been selected for use as standard or recommended[20,101] methods. The pyridine - pyrazolone colour development (Epstein) depends on the presence of a small amount of bis-pyrazolone and requires the preparation of a relatively unstable reagent. The pyridine - benzidine reaction with cyanogen halide (Aldridge's procedure) has the necessary sensitivity and stability, but has a distinct disadvantage in that the amine used is a well known active carcinogen. Also, there is some confusion as to the correct wavelength to use when measuring the developed colour in Aldridge's method. The problem is to improve on the existing methods by—

(*i*) the development of a one- or two-stage process that will give a colour with cyanide ions, hence reducing the amount of time necessary for determining cyanide ion, and probably permitting a suitable field method for determining microgram amounts of cyanide to be developed; or

(*ii*) use of a non-carcinogens in the König synthesis, preferably compounds that are more stable and have a greater molecular extinction coefficient than either pyrazolone or benzidine.

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The Analysis of ^{14}CO - $^{14}CO_2$ Mixtures by Gas-chromatographic Separation and Aqueous Solution Counting*

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A method is described for analysing carbon-14 monoxide - carbon-14 dioxide mixtures. Gas-chromatographic separation on a silica-gel column is used to determine both the percentage composition and the activity of each constituent. The carbon monoxide emerging from the column is oxidised to carbon dioxide and then frozen out in a cold trap to which sodium hydroxide solution has been added; the carbon dioxide component is isolated in a second cold trap. The activity of the solution from each trap is directly determined by scintillation counting in a cell containing a layer of plastic phosphor cemented to the inside of its base.

The rapid separation achieved by gas chromatography permits an analysis to be completed in 40 to 60 minutes, depending on counting time.

Results are presented for carbon-14 monoxide - carbon-14 dioxide mixtures covering a range of compositions.

In connection with tracer studies involving the use of carbon-14, it has been necessary to analyse numerous mixtures of carbon monoxide and carbon dioxide in which both constituents were labelled. For each mixture it has been required to determine the percentage composition and also the amount of activity present in each chemical form, so that the specific activity of each constituent could be calculated.

In our laboratories it has been customary for some years to analyse inactive gas mixtures containing carbon monoxide and dioxide by gas chromatography. For determining minor constituents, including carbon monoxide, in gases containing mainly carbon dioxide, the carbon dioxide has been removed by passage through soda-lime and the minor constituents separated on a molecular-sieve column.[1] When a determination of carbon dioxide has been required, its separation from carbon monoxide has been achieved with a silica-gel column. Such analyses can be completed in a few minutes, a matter of some importance if many samples are to be handled.

On the grounds of familiarity with the technique and the speed of analysis afforded, gas chromatography was adopted as the means of determining the percentage composition of the labelled gas mixtures. In addition, as determination of the individual activities of the carbon monoxide and carbon dioxide also necessitated their separation, it seemed obvious to take advantage of the separation of these constituents achieved in the gas-chromatographic column.

For measuring the activity of the chromatographically separated components, various procedures were available. The activity of the gas from the column could be continuously monitored by a suitable radiation detector, operated in conjunction with a ratemeter and recorder. Alternatively, the active components could be separately trapped out from the gas stream; counting in gas, liquid or solid forms could then be applied as desired. The different techniques employed in the gas-chromatographic separation of radioactive compounds have recently been reviewed by Adloff.[2]

For the work described here, continuous monitoring of the effluent gas would have required additional specialised counting equipment, and this approach was rejected in favour of isolation of the active components. The carbon monoxide, which was the first to emerge from the column, was oxidised to carbon dioxide with heated copper oxide and then frozen out in a cold trap, to which sodium hydroxide solution had initially been added. The carbon dioxide component was later isolated in a similar trap. After the traps had been warmed to room temperature, the activity of the solution in each trap was determined by a direct counting method.

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This procedure has proved satisfactory in use over 2 years. The speed of the gas-chromatographic separation is of particular advantage, and an analysis can be completed in 40 to 60 minutes, depending on the time required for counting.

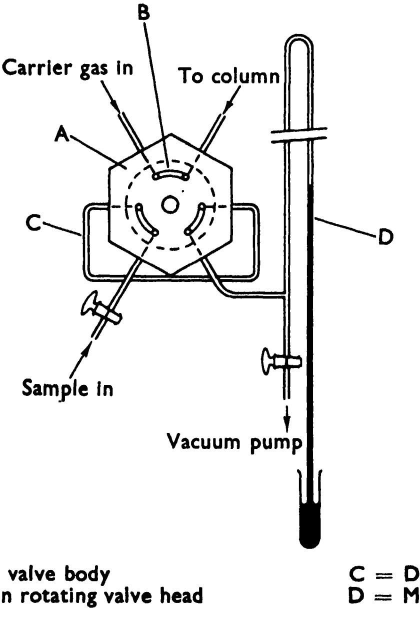
The development of this procedure involved investigations into the gas chromatography of active mixtures and into the counting of aqueous solutions containing carbon-14. These investigations are described below, preceded by an account of the apparatus and of the analytical procedure finally evolved.

APPARATUS

GAS-CHROMATOGRAPHIC APPARATUS—

The gas-chromatographic apparatus used was made in our laboratories and was similar to that previously described.[1] The analytical column was a 6-foot length of ¼-inch internal diameter stainless-steel tubing of 20 s.w.g. wall thickness, packed with 36- to 52-mesh silica gel that had been heated *in vacuo* before being packed into the column. A second packed column was used to obtain a parallel flow of carrier gas to serve as a reference gas for the detector. The columns were maintained at 100° C by an electrically heated steam-jacket. The carrier gas was argon, supplied from a cylinder and dried by passage over pellets of molecular sieve (Linde type 5A); the flow rate through each column was 30 ml per minute. The detector was a katharometer, made as previously described,[1] in which the sensitive elements were tungsten filaments. The signal from the katharometer, after attentuation by known factors, if required, was fed to a potentiometric recorder so that the heights of recorded chromatographic peaks could be measured. In addition, an attachment was made to the recorder so that the mechanical movement of the recorder pen produced, from a slide wire, a voltage proportional to the pen deflection; application of the voltage produced to an indicating integrating motor (Electromethods Ltd., Stevenage) permitted automatic reading of the "areas" of chromatographic peaks.

The gas-sampling system, incorporating a valve of the type previously described,[1] is shown in Fig. 1. With the valve in the position shown, the carrier gas passed directly to the column; at the same time the sample inlet was connected to a detachable sample loop of known volume and thence to a mercury manometer and to a vacuum pump. The sample loop was evacuated, and then the sample gas was admitted to the desired pressure. Rotation of the valve through 60° then allowed carrier gas to pass through the loop and sweep the sample on to the column, with minimal interruption in the flow of carrier gas.



A = Sampling valve body
B = Groove in rotating valve head
C = Detachable loop
D = Manometer

Fig. 1. Gas-sampling system, showing valve in sampling position

The gas samples to be analysed were usually in silica bulbs provided with a break-seal. To permit portions of the gas to be withdrawn without admitting air, the break-seal end of a bulb was connected by pressure tubing to a sampling head of the type shown in Fig. 2. The sampling head had a side-arm connected via a tap to the gas-sampling system described above. The end of the sampling head was closed with a rubber serum cap through which passed a steel wire with a shaped end-piece. In operation, the sampling head was evacuated simultaneously with the sample loop, and the tap between the sampling head and the gas-sampling valve was then closed. The steel wire was twisted to break the break-seal, and gas was admitted to the sample loop as required.

TRAPPING SYSTEM—

A two-way tap was fitted at the point where the gas stream from the analytical column emerged from the katharometer block. With the tap in one position, the gas passed directly to a trap cooled in liquid oxygen, to freeze out the carbon dioxide component. (Liquid nitrogen, if used, would condense the argon used as carrier gas.) With the tap in the other position, the gas passed over copper oxide at 500° C, to oxidise carbon monoxide to carbon dioxide, and thence to a second cold trap to isolate the carbon monoxide component. The copper oxide was in the form of a roll of copper gauze, oxidised by prior heating in oxygen and contained in a length of silica tubing of 3 mm internal diameter that was heated over a length of 4 inches by an external winding of Nichrome wire. To facilitate control of the apparatus, the volumes of tubing (glass and poly(vinyl chloride)) between the katharometer block and the traps were kept small, so that only a few seconds would elapse between emergence of a constituent from the column (as detected by the katharometer) and its arrival in the appropriate trap.

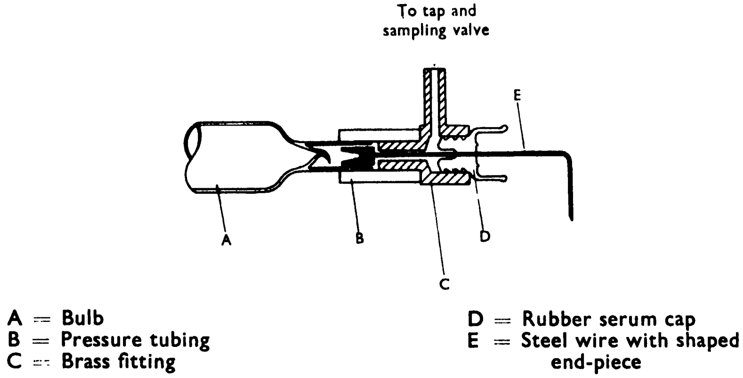


Fig. 2. Sampling head used with sealed bulbs

The traps, of borosilicate glass, were of the type shown in Fig. 3; their design was based on that of traps used to freeze out small amounts of carbon dioxide in the determination of carbon in steel by the "low pressure" method.[3] Sodium hydroxide solution was added to each trap before connection to the apparatus, so that the trapped active component was finally obtained as an alkaline aqueous solution. In use the traps were immersed in liquid oxygen up to the bottom of the joint. A vacuum grease was used on the joint of each trap, and, in addition, the joint was held in place with springs (not shown in Fig. 3). There was thus no risk of loosening the joint when the traps were held by the inlet and exit tubes for the purpose of manipulating the traps in and out of the liquid oxygen.

COUNTING CELL AND COUNTING EQUIPMENT—

The active aqueous solutions obtained in the traps on re-warming to room temperature were counted in a specially constructed cell, which is shown in Fig. 4. The main body of the cell was turned from 2-inch diameter Perspex rod. The base consisted of a disc of NE102 plastic phosphor, 0·005 inch thick (Nuclear Enterprises Ltd.), cemented to a $\frac{1}{16}$-inch thick Perspex disc; amyl acetate was used as the cementing solvent. The base was in turn cemented to the main body of the cell. The dimensions of the cell were chosen to permit use in an Ekco

N664 scintillation counter, operated in conjunction with an Ekco N530 scaler. It was found appropriate to use the same instrumental settings as used for scintillation counting of carbon-14 in toluene-2,5-diphenyloxazole solutions, namely: E.H.T., 1100 volts; gain, 1000 × ; discriminator setting, 17 volts.

METHOD FOR ANALYSING ^{14}CO - ^{14}CO$_2$ MIXTURES

PROCEDURE—

The bulb containing the gas sample to be analysed was connected to the sampling head (see Fig. 2), and the sample loop and sampling head were evacuated with the vacuum pump. The tap between the pump and the sampling system was then turned off and the system allowed to remain evacuated for several minutes to check for possible leaks.

Meanwhile, 5 ml of 0·1 N sodium hydroxide were placed by pipette into each of two dry traps, the traps were re-assembled and their taps closed. Each trap was dipped for a few seconds into liquid oxygen, removed and swirled, and the process was repeated until the sodium hydroxide solution had frozen in a thin layer on the wall of the trap tube. (Freezing in this manner avoided cracking the tubes.) The two traps were connected to the gas-chromatographic apparatus, immersed up to the bottom of the joint in liquid oxygen, and in turn purged of air with the flow of carrier gas from the column. The taps of the carbon dioxide

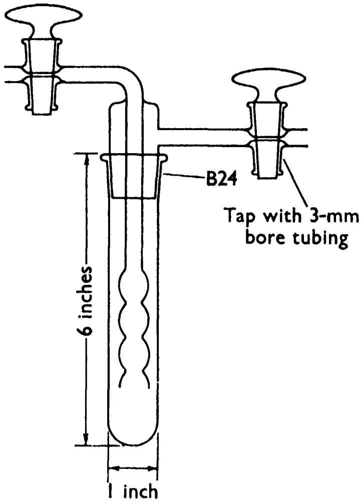


Fig. 3. Trap for freezing out carbon dioxide

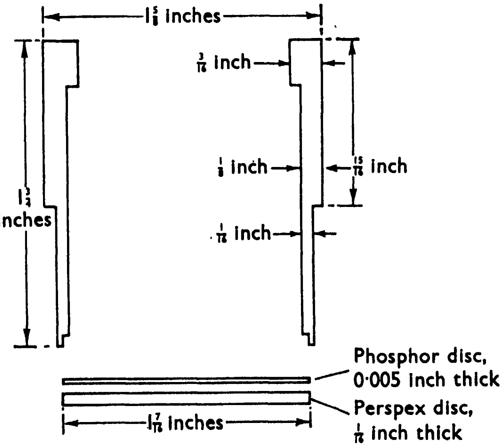


Fig. 4. Details of construction of counting cell

trap were then closed, and carrier gas was allowed to continue flowing through the carbon monoxide trap.

The break-seal of the sample bulb was then broken, and gas was admitted to the sample loop to the required pressure as shown on the manometer. (With a sample loop of volume about 1·5 c.c., sample pressures of about 200 mm of mercury were generally used, if sufficient sample was available). The sample gas in the loop was then injected into the column by operating the gas-sampling valve. The air temperature near the sample loop, assumed to be the same as that of the sample, was noted at this point.

Any carbon monoxide present in the sample was shown by a peak on the recorder chart occurring 1¼ minutes after injection of the sample. After a further 2 minutes, *i.e.*, 3 to 3½ minutes after injection, the taps were operated to divert the effluent gas from the column to the carbon dioxide trap, and the taps of the carbon monoxide trap were closed. The carbon dioxide peak was recorded about 4½ minutes after injection, and the gas stream was allowed to flow through the carbon dioxide trap for a further 2 minutes after the peak had been recorded.

While the carbon dioxide was being eluted, the exit tube of the carbon monoxide trap was connected to the vacuum pump via a capillary "leak." The exit tap of this trap was opened for a brief period, then closed and the vacuum connection removed. (It was necessary to remove most of the argon present in the trap, so that undue pressure would not result on subsequent re-warming of the trap to room temperature; the capillary leak restricted the flow of gas, so that small particles of solid carbon dioxide would not be blown out of the trap.) The trap was removed from the liquid oxygen and re-warmed to room temperature by immersion for a few minutes in warm water. Finally, the trap was shaken mechanically for 5 minutes to dissolve any carbon dioxide that was now in the gaseous form. The same series of operations was applied to the carbon dioxide trap.

For counting, most of the solution from a trap was transferred to the counting cell by means of a dry pipette (quantitative transfer is unnecessary, but dilution must be avoided). Fifty milligrams of a dye, Naphthalene Leather Carbon G.S. (Imperial Chemical Industries Ltd.), were added to the cell, and the solution was swirled to dissolve the dye. The cell was then transferred to the scintillation counter and the solution counted. Each count was corrected for the background count of the cell, determined by counting 5 ml of 0·1 N sodium hydroxide with 50 mg of dye in the cell. The background count was measured for each series of determinations, usually during the preliminary preparations of the chromatographic apparatus.

At least two, usually three, analyses were made on each gas sample. By having traps available, replicate analyses could be performed in rapid succession, the solutions from one analysis being counted during the chromatographic stage of the subsequent analysis. After use, the traps were well washed with water, then with acetone, and dried by drawing air through them. After each count the counting cell was washed well with water and dried with tissues; the cell was then ready for re-use.

CALCULATION OF RESULTS AND CALIBRATION—

The percentage of the minor constituent (usually carbon monoxide) was obtained by correcting the observed peak heights, peak areas, or both, to a standard sample pressure and temperature, then reading off the percentage from a calibration graph prepared by chromatographic separation of inactive gas mixtures of known composition. The percentage of the major constituent was usually obtained by difference. (Traces of nitrogen and hydrogen were sometimes present, but invariably in insignificant amounts.)

As discussed later, the counting technique used was such that the observed count rate, corrected for background, was proportional to the concentration of carbon-14 in the solution, regardless of the volume of solution added to the cell. The cell factor f (the net count rate given by a carbon-14 concentration of 1 μC per ml) was determined by counting a solution of sodium ^{14}C-carbonate of certified activity (nominally 1 μC per g) obtained from the Radiochemical Centre, Amersham.

If the solution from a trap gave a count rate r (corrected for background), then the total activity (in μC) in the trap was 5r/f, the total volume of the trap solution being 5 ml. The specific activity of a constituent, in μC per standard c.c., was then given by the expression—

$$\frac{5\mathrm{r}}{\mathrm{f}} \times \frac{760\,(273 + t)}{V \times p \times 273} \times \frac{100}{\mathrm{P}},$$

where P was the percentage of the constituent, and V in c.c., p in mm of mercury and t in degrees C were, respectively, the volume, pressure and temperature of the gas sample. Of these quantities, V was kept constant by using the same sample loop throughout the work.

COUNTING OF CARBON-14 IN AQUEOUS SOLUTION

Isolation of the active constituents as aqueous solutions was adopted because direct counting of the solutions appeared to offer the minimum amount of manipulation compared with counting in gas or solid form. The counting technique used was essentially that proposed by Jenkinson[4] and which appeared particularly appropriate to the work described here.

In the counting cell made by Jenkinson and in the cell used in this work, the scintillations detected by the counter occur in the thin layer of plastic phosphor that forms the inner side of the base of the cell. Because of the short range in water (< 1 mm) of the soft beta radiation of carbon-14, only the activity present in a thin layer of solution adjacent to the phosphor contributes to the observed count rate. This resembles the condition of "infinite thickness" in solid counting, from which it might be expected that the count rate would be proportional to the concentration of activity in the solution and be independent of the volume. However, Jenkinson observed some variation of count rate with different volumes of the same solution. He attributed this to variations in the extent to which light emitted upwards by the phosphor layer was re-directed to the counter by internal reflection in the solution. Jenkinson therefore added a dye to eliminate transmission of light through the solution; the count rate was then proportional to the specific activity of the solution and independent of the volume counted.

This property is an advantage in our work. The shape of the inner member of each trap, designed to give a large cold surface, was such that quantitative transfer of a solution from a trap would inevitably result in considerable dilution due to the addition of washings. By using Jenkinson's counting technique, any indefinite volume of the trap solution could be transferred to the cell for counting, provided, of course, that sufficient solution were added to cover the base of the cell.

Some experiments on this counting technique, relevant to the analysis of carbon-14 monoxide - carbon-14 dioxide mixtures, are described below.

PRELIMINARY EXPERIMENTS—

The first experiments were directed toward establishing the conditions under which consistent count rates could be obtained for a solution, independent of the volume counted. For this purpose, different volumes of sodium ^{14}C-carbonate solution, with and without dye, were counted. As recommended by Jenkinson,[4] the dye used was Naphthalene Leather Carbon G.S.

The results obtained (see Table I) confirmed Jenkinson's observations[4] that count rate varied with volume in the absence of dye, and that count rates independent of volume were obtained with 0·1 per cent. w/v of dye, this concentration not being critical. It was apparent that use of the dye only slightly reduced the counting efficiency.

TABLE I

PRELIMINARY EXPERIMENTS ON COUNTING OF $Na_2{}^{14}CO_3$ SOLUTION

Volume of solution counted, ml	Concentration of dye, % w/v	Count rate, c.p.s.
3	0	187·2
5	0	183·9
10	0	180·1
3	0·05	176·0
3	0·1	178·0
3	0·15	177·7
5	0·1	177·2
7·5	0·1	177·2
10	0·1	176·7

EFFECT OF CONCENTRATION OF ALKALI—

Jenkinson[4] reported that increased concentrations of inactive solute caused some reduction in count rate, presumably by a self-absorption effect. In the work described in this paper, the effect on count rate of different concentrations of alkali was investigated. A dilute solution of sodium ^{14}C-carbonate was counted after various amounts of inactive sodium hydroxide or carbonate solution had been added, the observed count rates being corrected for the resulting dilutions; the concentration of dye was 0·1 per cent. w/v in all solutions counted. The results in Table II showed a progressive reduction in corrected count rate with increasing concentration of alkali. It was however apparent that if 0·1 N solutions were used, appreciable relative variations in concentration would have negligible effect on count rate. For absorption of the carbon dioxide from the size of gas sample customarily used (1·5 c.c. at 200 mm pressure), 5 ml of 0·1 N sodium hydroxide would provide a substantial excess, and 0·1 N sodium hydroxide was adopted as the medium for counting.

TABLE II

EFFECT OF INACTIVE ALKALI CONCENTRATION ON COUNT RATE OF AN $Na_2{}^{14}CO_3$ SOLUTION

Alkali added	Concentration of added alkali in solution counted, N	Count rate, % of count rate without added alkali
Sodium hydroxide	0·10	99·8
	0·17	99·2
	0·27	98·8
	0·50	98·8
	1·0	97·8
Sodium carbonate	0·10	99·8
	0·18	99·4
	0·33	98·9
	0·67	97·5
	1·0	96·9

CALIBRATION AND COUNTING EFFICIENCY—

Calibration of the cell has been effected, as described on p. 765, by counting a sodium ^{14}C-carbonate solution of certified activity. The first cell used in this work gave, on first calibration, a cell factor of 330 c.p.s. (the count rate for 1 μC of carbon-14 per ml). A second cell gave a similar factor. As the same count rate is obtained with different volumes of the same solution, the counting efficiency achieved depends on the volume of solution counted. With 5 ml of solution, the cell factor quoted above corresponds to a counting efficiency of 0·2 per cent. This relatively low efficiency was no disadvantage in the work described here, the main virtue being the simplicity of the counting technique. The gas samples to be analysed contained several μC of carbon-14 per standard c.c., so that sizable count rates, *e.g.*, of the order of 100 c.p.s. for the main constituent, were usually obtained.

With a cell in almost daily use, it has been found that the base tends in time to become slightly opaque, possibly owing to weakening of the adhesion between the plastic phosphor layer and the underlying Perspex. Possibly for this reason the counting efficiency of a cell decreases in time, and it is advisable to re-calibrate a cell every few months.

Jenkinson stated that high background count rates with his cell could arise either from undue exposure of the cell to light or from use with neutral or acid solutions, hence his recommendation that all solutions be made alkaline with sodium hydroxide before counting.[4] In one cell used by us, the latter effect was observed in attempting to count a solution containing carbon-14 labelled sucrose. The former effect has not been observed; handling of the cell in daylight or normal lighting conditions has invariably given background counts indistinguishable from that of the scintillation counter with no sample present (0·5 to 0·6 c.p.s.) The precaution has, however, been taken of storing the cell in the dark when not in use.

GAS CHROMATOGRAPHY OF THE LABELLED GAS MIXTURES

In developing the gas-chromatographic part of the procedure described, particular attention was directed to assessing the efficiency of the trapping system, to examining the possibility of "trailing" of active constituents from the column and to determining the precise volume of gas sample analysed. These points are discussed below.

EFFICIENCY OF THE TRAPPING SYSTEM—

The efficiency of the cold traps for freezing out carbon dioxide was tested by putting samples of carbon-14 dioxide through the gas-chromatographic procedure, two traps in series being used. In two runs on slightly different amounts of sample, count rates of 48·3 and 45·5 c.p.s. were obtained for the solutions from the first trap, corresponding, respectively, to

2·56 and 2·57 μC per standard c.c. for the specific activity of the gas; in each test the second trap yielded no detectable activity ($<$ 0·1 c.p.s.).

These and other results showed that a single cold trap gave quantitative removal of carbon dioxide from the steam of carrier gas. However, to ensure complete removal of the carbon monoxide component, it was also necessary to achieve its complete oxidation to the dioxide on passage through the heated copper oxide. The efficiency of the oxidation process was checked by putting samples of inactive carbon monoxide through the gas-chromatographic process and collecting the carrier gas emerging from the copper oxide tube during the period when the carbon monoxide was being eluted from the column. Analysis of the collected gas showed that quantitative oxidation of the carbon monoxide was being obtained.

EXAMINATION FOR TRAILING EFFECTS—

Under the conditions employed in the method, the peaks of carbon monoxide and dioxide occurred, respectively, at 1¼ and 4½ minutes after injection of the sample. For the first 3 or 3½ minutes after injection, the gas from the column was passing into the carbon monoxide trap; thereafter, until 2 minutes after the end of the carbon dioxide peak (as observed on the recorder chart) the gas was passing into the carbon dioxide trap.

With this system, any trailing, *i.e.*, the slow release of a constituent from the column after the main peak had passed, could result in incomplete recovery of the activity. In addition, trailing of the carbon dioxide could result in activity from this constituent being included in the carbon monoxide fraction of a succeeding analysis. It was therefore important to ascertain whether or not trailing occurred to any significant extent.

With a comparatively new column being used, a sample of carbon-14 dioxide was passed through the gas-chromatographic apparatus, the carbon dioxide trap being replaced at intervals after the peak had been recorded. Only an extremely small amount of activity, negligible when compared with the total amount present, was recovered after the normal period of collection (see Table III). A similar test with carbon-14 monoxide, though less sensitive because of the smaller amount of activity used, showed no evidence of trailing (see Table III).

It was apparent from these results that trailing was unlikely to be a significant source of error when a fairly new column was used. It is, however, well known that trailing may become more evident with continued use of a column. In this and related work it has been found that trailing and other adverse effects may necessitate renewal of the columns after about 6 months. (The life of a column appears to be largely a function of time, rather than of frequency of use, possibly because of our practice of maintaining a slow flow of carrier gas through a column when it is not in use.) It may be of interest to note that chromatography of active gases can be made an extremely sensitive means of detecting trailing.

TABLE III

EXAMINATION FOR TRAILING EFFECTS

Sample used	Period of collection of active constituent	Count rate of trap solution corrected for background, c.p.s.
Carbon-14 monoxide ..	Up to 2 minutes after CO peak	13·4
	2 to 5 minutes after CO peak	<0·1
	5 to 10 minutes after CO peak	<0·1
Carbon-14 dioxide ..	Up to 2 minutes after CO_2 peak	159
	2 to 5 minutes after CO_2 peak	0·2
	5 to 10 minutes after CO_2 peak	<0·1

DETERMINATION OF SAMPLE VOLUME—

The same sample loop, of measured volume 1·42 c.c., was used for all determinations. However, this volume did not represent the total volume of gas sample analysed, a small additional amount of gas being present in the connections inside the gas sampling valve (see Fig. 1). This additional volume was known, from the construction of the valve, to be about 0·15 c.c. For determining the percentage composition of gases, the presence of this additional volume of sample was irrelevant, as the same total volume of gas would be used both in analysis and in calibration with gases of known composition. However, to permit calculation of the specific gravity of constituents, in μC per standard c.c., it was essential to know the precise volume of gas sample analysed.

The additional volume of gas in the valve was determined indirectly. Samples of active and of inactive carbon dioxide were submitted to the chromatographic process, with the use of both the normal sample loop and a small loop of volume (0·253 c.c.) similar to that in the valve. At a given sample pressure, the ratios of peak areas and of amounts of activity found were assumed to be proportional to the ratio of sample volumes used, *i.e.* $(1{\cdot}42 + x)/(0{\cdot}253 + x)$, from which x, the volume of gas in the valve, could be calculated. Four such determinations gave values of 0·15, 0·15, 0·18 and 0·19, the mean value of 0·17 c.c. being taken. This value was consistent with the expected volume of about 0·15 c.c.

With the normal loop, of volume 1·42 c.c., the total sample volume was therefore taken to be 1·59 c.c. This value was thought to be accurate to within about 1 per cent. Because of the expected accuracy of the activity measurements, a much greater accuracy in measuring the precise sample volume would have been superfluous.

RESULTS

The results obtained on a sample of carbon-14 dioxide are shown in some detail in Table IV, in which sample pressures and temperatures and also the observed count rates are listed.

TABLE IV

RESULTS ON A SAMPLE OF $^{14}CO_2$

Sample pressure, mm of Hg	Temperature, °C	Count rate of trap solution, c.p.s.	Count rate corrected for background, c.p.s.	Specific activity of CO_2, μC per standard c.c.
151	30	48·9	48·3	2·56
142	30	46·1	45·5	2·57
92	31	28·8	28·2	2·46
135	26	44·2	43·6	2·55
101·5	31·5	32·6	32·0	2·54

This particular sample was of lower specific activity than normally used, giving rise to lower count rates than normally observed. The sample, in which no carbon monoxide was detected, was subjected to the analytical process primarily to assess the precision obtainable in that part of the process involving the trapping of the carbon dioxide and the measurement of activity.

To illustrate the precision obtained in the analysis of mixtures, in which an additional possible source of error arises from the determination of percentage composition, the results of analyses of a typical series of samples are shown in Table V. Each figure quoted for the

TABLE V

RESULTS FOR A TYPICAL SERIES OF ^{14}CO - $^{14}CO_2$ MIXTURES

Sample number	Carbon monoxide present, %		Specific activity of CO (A), μC per standard c.c.		Specific activity of CO_2 (B), μC per standard c.c.		Ratio of specific activities B/A
1555	30·5 31·3	30·9	5·2 5·2	5·2	5·29 5·31	5·3	1·02
1552	14·0 13·7 13·8	13·8	5·65 5·85 5·75	5·75	6·05 6·03 5·96	6·0	1·04
1553	10·1 9·9 10·0	10·0	5·95 5·9 5·75	5·85	6·14 6·19 6·10	6·15	1·05
1547	7·9 7·75 7·8	7·8	5·9 6·25 6·3	6·15	6·21 6·25 6·38	6·3	1·02
1549	7·1 7·2 7·15	7·15	5·85 5·95 6·0	5·95	6·31 6·13 6·16	6·2	1·04
1546	6·6 6·65 6·65	6·65	5·8 6·1 6·2	6·05	6·28 6·30 6·19	6·25	1·03
1548	5·65 5·55	5·6	6·0 6·0	6·0	6·50 6·41	6·45	1·07

percentage carbon monoxide was the mean of the two values deduced from the height and from the area of the peak; for calculating the specific activity of the carbon dioxide, the percentage of carbon dioxide was taken by difference.

The gas samples referred to in Table V were from reactor-irradiated bulbs that had originally contained only graphite and labelled carbon dioxide, the carbon monoxide found being a product of the radiation-induced reaction between graphite and carbon dioxide. The carbon monoxide, being partly derived from the carbon of the unlabelled graphite, would be produced at a specific activity lower than that of the carbon dioxide; in addition, interchange of activity between the two gaseous constituents would arise from the radiation-induced exchange reaction—$^{14}CO_2 + CO \rightleftharpoons {}^{14}CO + CO_2$. At equilibrium, the ratio of specific activities of the two constituents is equal to the equilibrium constant for the exchange reaction. The results in Table V show essentially constant values for the ratio of specific activities of carbon dioxide and carbon monoxide, the mean ratio being 1·04. This figure is in excellent agreement with the value of 1·03 ± 0·008 for the equilibrium constant of the exchange reaction, reported by Stranks.[5]

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The Determination of Oxygen in Vacuum-melted Steels, Molybdenum and Single-crystal Silicon by Vacuum Fusion

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The equipment and techniques used for determining oxygen in vacuum-melted steels, molybdenum and single-crystal silicon by vacuum fusion, at levels down to less than 1 p.p.m., are described.

The oxygen content of steels at levels of 115 p.p.m. and 6 p.p.m. has been determined with coefficients of variation of 2·3 and 5·2 per cent., respectively. Results on sintered and arc-cast molybdenum with oxygen levels of 42 p.p.m. and 2 p.p.m. showed coefficients of variation of 2 and 8 per cent., respectively. Experiments with zone-refined molybdenum indicated that the oxygen content consisted of a residual surface contamination of 0·8 μg per sq. cm with an internal oxygen content of less than 0·2 p.p.m. With vacuum grown single-crystal silicon a coefficient of variation of 10 per cent. at a level of 17 p.p.m. was obtained on a limited sample weight of 0·5 g.

A COMPREHENSIVE account of the vacuum fusion method of analysis and its applications has been given by Sloman.[1 to 7] In recent years developments of the technique have been concentrated chiefly along two lines of research, *viz.*, analyses of the rarer metals (beryllium, molybdenum, tantalum, titanium, tungsten, etc.) and greater accuracy and precision in determining low levels of oxygen content. Progress in the latter field, however, still lags behind metallurgical requirements, and dissatisfaction has been expressed[8,9] at the lack of precision and accuracy obtainable at low levels of oxygen content (0 to 20 p.p.m.) in the vacuum-fusion analysis of steel and molybdenum.

Little evidence is available in the literature for reproducibilities of determinations of oxygen of below 100 p.p.m. Lassner and Wölfel[10] have obtained a coefficient of variation of 6·1 per cent. on six samples of sintered molybdenum with a mean value of 30 p.p.m., and Mallet and Griffith[11] have demonstrated the internal consistency of the method below 40 p.p.m. to within ±1 p.p.m. on samples of molybdenum spiked with molybdenum powder. McDonald and Fagel[12] have determined oxygen in molybdenum by vacuum extraction at 2000° C and by vacuum fusion in a tin - iron bath. By vacuum extraction they obtained a mean of 7·6 p.p.m. on eleven samples with a coefficient of variation of 36 per cent.; by vacuum fusion the coefficient of variation was 21 per cent. on a mean of 7·3 p.p.m. on four samples of the same material.

Many of the results quoted indicated that the method was reliable for steel, although, recently, Pearce and Masson[13] working on the method of isotopic dilution have produced evidence suggesting that the results obtained by vacuum fusion analysis of steels were seriously in error below 200 p.p.m. owing to adsorption of the evolved gases on the cooler parts of the apparatus. However, their experiments do not appear to be conclusive, and their conclusions conflict with the main body of evidence[6,7,14,15] and our experience. Recent work by Coleman[16] on fast neutron-activation analysis has also confirmed the reliability of vacuum-fusion analysis on two of the samples quoted by Pearce and Masson.

The object of the work described here was to develop the technique for determining oxygen in vacuum-melted steels, sintered, arc-cast and zone-refined molybdenum and single-crystal silicon, all with an oxygen content below 100 p.p.m. It was considered that, to meet the needs of the metallurgist, a method having a coefficient of variation of not more than 10 per cent. was required at levels down to 1 p.p.m.

APPARATUS

GENERAL—

The vacuum fusion apparatus (see Fig. 1) was similar in its basic design to that described by Gray and Davis.[17] Use was made of Sloman's design of crucible and furnace assembly and the principles of Ransley's low pressure analysis system.[18,19] The measuring system has been described previously by Bacon[20] and was designed to measure volumes of gas from 0·005 to 2·0 ml with a precision of ±1 per cent. by using a McLeod gauge and a series of expansion volumes that permitted the pressure to be kept within that of the maximum precision of the McLeod gauge by varying the capacity of the collecting volume.

MEASURING AND ANALYTICAL SYSTEMS—

The minimum collecting volume normally used was 917 ml, although the smaller volume of 692 ml could be used by excluding the McLeod gauge and using the Pirani for pressure measurements. This was done for determining the blank rate or for analysing small samples (below 2 g) with extremely low contents of gas (below 2 p.p.m.). The collecting volume could be increased to 21 litres in steps, increasing each time by a factor of approximately 2, by means of expansion volumes. The collecting volumes were calibrated volumetrically by adding water and checked by means of expansion ratios. The results agreed to within 1 per cent.

Two pressure gauges were used, a McLeod for making accurate measurements and a Pirani for making rapid measurements, determining precise pressure ratios and registering the gas evolution and so following the course of the reaction. The McLeod gauge had two pressure ranges, 0 to 70 × 10^{-3} torr and 0 to 300 × 10^{-3} torr. The fine scale had both linear and square-law calibration. The linear range, which was based on a compression ratio of 2000 to 1, had 1-mm graduations corresponding to 0·5 × 10^{-3} torr pressure increments. Repeated observations of the pressure with this scale showed the reading error to be no more than 0·5 mm (0·25 × 10^{-3} torr). Used in the range 25 to 70 × 10^{-3} torr the gauge had, therefore, a maximum reading error of 1 per cent.

The Pirani gauge had a normal G.E.C. gauge head and was operated with a potential of 4 volts across the bridge. The out-of-balance current was recorded on a milliammeter with 1 mA full-scale deflection and an eye sensitivity of 1 μA. Three pressure ranges were provided by means of shunts. The first two were linear over their full ranges, which were 0 to 7 × 10^{-3} torr and 0 to 35 × 10^{-3} torr, and the third scale, which was non-linear, was used merely as a guide for pressure in excess of 35 × 10^{-3} torr. Pressure ratios, obtained by readings of near full-scale deflection on the linear scales, had a theoretical reproducibility of 0·1 per cent., and results close to this were obtained in practice. For readings less than full scale, correspondingly larger percentage variations were of course obtained. In practice, the coarser scale (0 to 35 × 10^{-3} torr) was preferred, as slight pressure changes due to adsorption and mechanical disturbances had less effect at the higher pressures.

NEW FEATURES AND IMPROVEMENT OF TECHNIQUE AND DESIGN—

A summary is given below of the new features introduced and modifications in technique and design.

(*a*) The furnace head was re-designed in Pyrex glass and a fast mercury diffusion pump (rated at 30 litres per second) joined into the head with wide bore tubing (greater than 45 mm internal diameter) to allow rapid removal of the gases from the furnace tube. A positive means of guiding samples into the crucible was provided, and the optical flat was largely screened from the crucible to avoid blackening by carbon and metallic films.

(*b*) An ultra-pure grade of graphite was used (208 spectrographic, obtainable from Le Carbone (Gt. Britain) Ltd., 64 Finsbury Pavement, London, E.C.2). This allowed more rapid de-gassing and lower ultimate blank rates than could be obtained with normal "pure" graphite.

(*c*) A vacuum lock was incorporated to replace the mercury lift used in earlier work. This provided a rapid and convenient means of introducing all types of samples directly into the high-vacuum system. The lock was a scaled-up version of that described by Parker,[21] with the addition of vacuum connections to the gaps between the

O-rings. Little air entered the system with the samples, and no change was detected in the furnace blank rate after the lock had been used.

(*d*) A silica furnace tube in which samples could be heated to 1000° C was attached to the sample storage arm. Pre-heating removed much of the surface contamination and also greatly reduced the hydrogen content of samples. This allowed a more precise and accurate determination of the oxygen content, particularly on samples containing relatively large amounts of hydrogen or having an extremely low oxygen content.

(*e*) A gas "doser" was connected to the system to introduce different amounts of a gas of known composition into the apparatus to study gettering problems. This apparatus consisted of a 180-ml glass bulb connected to two smaller bulbs of 0·71- and 0·18-ml capacity, by good quality vacuum taps. The secondary volumes were so arranged that either 1/1000 or 4/1000 of the content of the main bulb could be fractionated into the vacuum-fusion system.

(*f*) A series of eight storage bulbs, each of about 110-ml capacity, was connected to the gas-collection system through a mercury diffusion pump. Gas samples could thus be stored during a run and analysed at a more convenient time. A silica furnace tube was also connected to this diffusion pump so that the equipment could be used for determining hydrogen by vacuum extraction.

(*g*) The vacuum-fusion furnace tube was air cooled. This system replaced the water cooling that had been used previously. Little difference was noticed between the two methods, but it seemed preferable to allow the walls of the furnace tube to reach the higher temperatures attained by air cooling as they would be less likely to absorb gases.

(*h*) Two types of induction heater were used; one of 8 kW operating at a nominal frequency of 750 kc/s and the other of 17 kW at a nominal frequency of 5 kc/s. The latter unit was preferred as the lower frequency induced a stirring action in the melt giving a more rapid evolution of gas. Low blank rates were obtained more consistently with this lower frequency as there was no tendency for the graphite powder to pick up induced currents as occurred with the higher frequency, which at times gave rise to "hot spots" and consequent higher blank rates.

(*i*) High-vacuum taps of good quality were used throughout the system in preference to mercury cut-offs. A liquid nitrogen trap was kept on the analytical system while the apparatus was in use, and this served to remove grease vapours, water, carbon dioxide and other condensable substances from the system.

(*j*) A series of four expansion volumes as from 0·7 to 11 litres was incorporated into the collecting system so that the volume could be adjusted to bring the pressure measurements within the region of linearity of the Pirani gauge and close to the pressure of maximum precision of the McLeod gauge.

EXPERIMENTAL

DE-GASSING—

The method of packing the graphite powder and the initial application of heat to the crucible were found to be critical operations, on which the successful de-gassing and the final blank rate were dependent.

Graphite powder (less than 325 mesh) was first compacted in the narrow extension of the furnace tube, to a depth of 0·5 inch. The crucible assembly was then placed in position and held centrally in the tube by means of a jig that fitted into the throat of the crucible. Graphite powder was packed around the crucible as loosely as possible. The furnace tube was then sealed into the head with vacuum wax, the induction heater and air cooling system were switched on, and the crucible was heated to approximately 1400° C. (All temperatures were measured with a disappearing filament optical pyrometer and are uncorrected.) The pressure in the furnace was then reduced by means of the "rough" vacuum pump at a rate of 3 cm per minute to a pressure of 10^{-1} torr, after which the furnace diffusion pump was switched on and the pumping routed through the analytical system. The temperature of the crucible was then raised to 2200° C and the apparatus allowed to de-gas for 3 hours.

BLANK RATES—

The procedures outlined above gave a final blank rate of approximately 1 μl per minute at 2200° C. When the temperature was dropped to 1750° C the blank rate fell to 0·3 μl per minute. Lower blank rates could be obtained by a more prolonged de-gassing period, but were difficult to maintain during a "run." A blank rate of 0·3 to 1 μl per minute was therefore accepted as a working level and was usually maintained if the samples added were of low gas content.

The "blank" gas was fairly constant in quantity and composition, and the results were corrected for this additional gas if it amounted to more than 1 per cent. of the total gas collected from the sample. A typical analysis of "blank" gas gave 75 per cent. of carbon monoxide, 7 per cent. of hydrogen and 18 per cent. of nitrogen.

GAS ANALYSIS—

A sample of the gas to be analysed was isolated in the smallest volume (the analytical section) at a pressure of 25 to 30 × 10^{-3} torr (*i.e.*, nearly full-scale deflection on the coarse Pirani scale). To obtain this reading the pressure of the gas sample could be either reduced by appropriate expansion or increased by pumping the gas from the volumes in which it was contained into the gas storage bulbs and then back into the analytical section. Small adjustments were made by varying the level of the mercury in the McLeod gauge.

The first stage of the analysis was carried out by exposing the gas to Hopcalite reagent contained in the small bulb attached to the analytical system. This oxidised the carbon monoxide to carbon dioxide, which was then removed from the system by condensation in the cold finger (−196° C). The palladium thimble was then heated to 450° C and the hydrogen allowed to diffuse out. The gas remaining was assumed to be nitrogen. The percentages of the three gases present were calculated from the pressure changes on the Pirani gauge. For this calculation the recorded decrease in pressure due to hydrogen was multiplied by a factor of 0·65, since the Pirani has a higher sensitivity for hydrogen than for carbon monoxide and nitrogen, which have similar sensitivities.

A volume of 0·010 ml at S.T.P. was taken for each analysis, 2 minutes were allowed for removal of carbon monoxide and 1·5 minutes for hydrogen diffusion. The time taken for a complete analysis was 4 minutes.

The precision of the analytical system was tested in practice on gas mixtures of known composition, and the over-all coefficient of variation for oxygen was found to be less than 1 per cent. when carbon monoxide formed more than 50 per cent. of the gas collected.

USE OF TIN AS AN "ANTI-GETTER"—

The use of tin as a flux or to overcome gettering is well known, and our experience has confirmed the efficacy of this technique.

The addition of tin (0·1 g) at intervals has been found to overcome the effect of gettering during vacuum fusion analysis, even with steels containing relatively large amounts of manganese, copper, vanadium and other alloy additions, possibly by blanketing the deposited films. Other theories have been advanced,[22] but the mechanism is still somewhat obscure.

ASSESSMENT OF ACCURACY

Direct tests of the accuracy of the method at low levels of oxygen, which were of interest, by preparing and analysing samples of known oxygen content were not considered feasible. Spiking samples with known weights of oxide, although not simulating exactly the conditions encountered in the analysis of a sample, gave an assessment of other stages of the process and was some indication of the accuracy to be expected. In this method the choice of a suitably prepared metal envelope having a low oxygen content, to contain the additions, minimised the objections to this type of evaluation.

EXPERIMENTS ON SAMPLES WITH KNOWN AMOUNTS OF OXIDE ADDED—

Two series of experiments were carried out, one with a steel capsule as a container for silica and the other with nickel foil to contain additions of tantalum oxide. The mean oxygen content of the capsules and the nickel foil was determined by carrying out ten determinations on each material.

Small fragments of drawn silica filament were used for the first series of experiments, and tantalum oxide, prepared from pure tantalum wire ignited at 1000° C in air under conditions in which the form of the wire was retained, was employed for the second series. The increase in weight of the tantalum was found to be that required by the reaction Ta → Ta_2O_5. The results of these experiments are shown in Table I.

TABLE I

RESULTS

Oxide added	Amount of oxide added, μg	Oxide content determined by vacuum fusion, μg	Equivalent to oxygen in metal on 10-g sample, p.p.m.	Error, %
Silica	92	99	4·9	+7·6
	159	156	8·5	−1·9
	146	153	7·8	+4·8
	194	180	10·4	−7·2
Tantalum oxide ..	260	294	4·7	−13·1
	163	173	2·95	+6·2
	325	316	5·9	−2·7
	280	254	5·1	−9·3
	296	315	5·35	+6·4
	190	215	3·45	+13·1
	220	197	4·0	−10·4
	315	331	5·7	+5·1
	243	263	4·4	+8·3

With the exception of two results the recovery of oxygen from the samples was within about ±10 per cent. of the amount added. The gas evolved from samples of approximately 27 μg of tantalum oxide and 94 μg of silica would be equivalent to that evolved by a 10-g sample of metal containing approximately 5 p.p.m. of oxygen.

OTHER INDICATIONS OF ACCURACY—

The considerations listed below, although not constituting any proof of accuracy, do serve to give added confidence in the validity of the results obtained.

1. At low oxygen levels samples were analysed at two sample weights, usually 10 g and 1·5 g. Agreement between the results indicated satisfactory sample preparation and absence of interference by surface oxide.
2. Similar results were obtained under widely different conditions, for example, molybdenum fused in an iron bath at 1750° C and without a bath at 2200° C (see Table V).
3. Single-crystal silicon gives an absorption band in the infrared at 9 μ due to the oxygen content. The ratio of the mean oxygen contents of the vacuum fusion results for the two silicon samples (see Table VI) was 1·39. This compared reasonably well with the ratio of 1·47 obtained from infrared measurements of the 9-μ band in the two samples.

The results of the experiments detailed above constituted reasonable grounds for considering that the vacuum-fusion process gives results for low levels of oxygen in metals which are accurate within the limits of the standard deviations obtained.

SOURCES OF ERROR IN VACUUM-FUSION ANALYSIS

Consideration was given to possible sources of error in vacuum fusion analysis, and for this purpose the procedure was broken down and examined in four categories.

SAMPLE PREPARATION—

Two possible sources of error were apparent in this procedure, namely errors due to oxide film remaining on the samples, and errors due to removal of internal oxygen by outward diffusion as carbon monoxide during the pre-heating.

Although the possibility of outward diffusion of oxygen had to be considered, it was not thought that this was likely to occur to any great extent under the pre-heating conditions used, and this was shown to be true.

A collection of the gas evolved during the pre-heating period showed that approximately 1·5 μg of oxygen per sq. cm was given off from the samples. Most of this gas was collected during the first 5 to 10 minutes, and heating for more prolonged periods (up to 1 hour) did not produce any appreciable increase in the amount of carbon monoxide collected. Some results obtained on steel and molybdenum are shown in Table II.

TABLE II

EFFECT OF DIFFERENT PRE-HEATING TIMES

Material	Pre-heating time, minutes	Number of determinations	Mean oxygen content, p.p.m.	Standard deviation, p.p.m.	Weight of sample taken, g	Surface area of sample, sq. cm	Approximate amount of oxygen removed from surface, μg per sq. cm
Steel	0	6	6·7	0·25	2	2·2	1·2
	10	6	5·4	0·17	2	2·2	1·2
Molybdenum ..	0	5	4·6	1·2	1·5	1·5	1·9
	10	6	2·7	0·6	1·5	1·5	1·9

The extent of any remaining oxide should be shown up by differences in results obtained on large and small samples of the same material, but the simplicity of results obtained on vacuum-melted steel at the lowest level available (see Table IV), showed that this was not significant. For molybdenum, however, there was evidence (see Table V) of a remaining oxide contamination of about 0·8 μg per sq. cm.

FUSION AND GAS EVOLUTION—

Thermodynamic considerations[6,23] have shown that the solubility of oxygen in an iron bath saturated with carbon at temperatures above 1650° C and in contact with a pressure of carbon monoxide below 10^{-3} torr, is negligible. The similarity of results obtained under different conditions (see Table V) would seem to confirm this conclusion, which has been accepted as the basis of all vacuum-fusion work.

GAS COLLECTION AND ANALYSIS—

The completeness of the gas recovery was checked by using the gas "doser" filled with carbon monoxide to a pressure of 15 cm. This was attached to the sample entry system as shown in Fig. 1. The results are shown in Table III.

TABLE III

RECOVERY OF CARBON MONOXIDE UNDER DIFFERENT CONDITIONS

The gas was collected for 1 minute

Amount of iron as charge in crucible, g	Temperature, °C	Carbon monoxide added, ml	Tin added, g	Carbon monoxide recovered, ml
0	1950	$0{\cdot}067_1$	0·0	$0{\cdot}067_0$
20	1950*	$0{\cdot}067_0$	0·0	$0{\cdot}049_4$
20	1950†	$0{\cdot}067_0$	0·1	$0{\cdot}066_7$

* Temperature maintained for 2 hours.
† Temperature maintained for 2 hours 20 minutes.

These results gave a strong indication that, provided the crucible was not allowed to run for prolonged periods without additions of tin being made, carbon monoxide was recovered quantitatively from the region of the crucible.

So far it has been tacitly assumed that any oxygen present in the samples would be converted to carbon monoxide. This was a reasonable assumption, as the monoxide is by far the most stable oxide of carbon under the conditions of fusion. To confirm that the dioxide was absent, the gas evolved from samples of steel was collected with a solid carbon dioxide - acetone cold trap in position in place of liquid nitrogen. After the gas had been collected this trap was replaced by liquid nitrogen, when in all instances a pressure drop not exceeding 1 per cent. of the total pressure was recorded. Part of this decrease was due to the temperature effect of the liquid nitrogen, but the experiment indicated that, if carbon dioxide was present, it consisted of less than 1 per cent. of the total gas collected (80 per cent. of carbon monoxide). This agrees with the conclusion of other workers[24] who have found that carbon monoxide, nitrogen and hydrogen form at least 99 per cent. of the gas evolved.

HOMOGENEITY OF SAMPLE—

To determine the effect of possible variations in the method it was necessary to have homogeneous samples. Several secondary steel standards were available from the British Iron and Steel Research Association and covered the higher part of the range 40 to 150 p.p.m.; the results obtained on these agreed closely with those of other analysts. However, an investigation of the oxygen distribution in one such sample (B.B.L.) showed a considerable concentration gradient of oxygen from the centre to the outside of the bar, and for this reason sections taken from ingots were preferred for determining the standard deviation of the method. Sections 2 inches × 2 inches × 1 inch were taken from near the centre of the ingot, the 1-inch dimension being along the longitudinal axis. Non-uniformity in the sample would of course increase the standard deviation obtained, but the similarity of the coefficient of variation for the different sample weights of A437 suggested that this was not a factor, for this sample at least. An independent check of homogeneity was possible only with the samples of silicon, when the coefficient of variation determined by infrared methods was less than 3 per cent. Arc-cast molybdenum samples were taken from near the centre of the ingots, but for sintered and zone-refined material ⅝-inch bar was used.

METHOD

PROCEDURE FOR STEEL—

Various types of steels were analysed successfully, including alloys containing 20 per cent. of manganese. The steels for which the apparatus was developed, however, were vacuum-melted R.A.R.D.E. high tensile steels, usually having an oxygen content between 5 and 15 p.p.m. and containing various alloying elements such as copper, manganese and vanadium in amounts up to 3 per cent. of each element.

Samples were usually received in cubes of side 1 inch taken from near the centre of the ingot. For analysis these were fabricated into right cylinders with hemispherical ends 1 cm in diameter, 2 cm in length and weighing 10 g. An alternative type of sample was a cube of side 6 to 7 mm weighing 1·5 to 2 g. The cylindrical samples were polished with carborundum cloth; the surfaces of the cubes were cleaned with a fine file. When clean, the samples were weighed immediately and introduced into the high vacuum by means of the vacuum lock. Usually the smaller type of sample was used; they were easier to prepare and clean, and more samples could be analysed in one run.

When they had been placed in the sample loading compartment, the samples were moved magnetically into the silica pre-heating furnace tube which held 5 of the large or 15 of the small samples. When the samples were in position, the resistance furnace was moved over the silica tube and the temperature of the samples raised to 1000° C. Fifteen minutes were allowed for the 10-g samples to reach this temperature and 10 minutes for the small samples.

Before the samples were fused, a 10-g slug of vacuum-melted steel was placed, together with 0·1 g of tin, in the previously de-gassed crucible. The steel added with the tin prevented spluttering of the latter and also assisted the rapid melting of the subsequent samples. Analysis was then begun.

The gas evolved from each sample was collected for 2 minutes. This was found to be sufficient time, since the main evolution of gas, as shown on the Pirani gauge, occurred within the first 30 seconds, and at the end of 2 minutes the evolution of gas had usually dropped to the level of the blank rate. The gas collected was measured and analysed. After the analysis of every third sample, 0·1 g of tin was added to prevent gettering. Samples were analysed at the rate of one every 10 minutes, which included the time required for blank determinations.

PROCEDURE FOR MOLYBDENUM—

Samples of molybdenum were received as sections of vacuum arc-melted ingots or as sintered or zone-refined rods of approximately 1 cm diameter. These were usually machined into right cylinders of length 2 cm and diameter 1 cm with hemispherical ends (weight 13 g). The dimensions were varied occasionally to give samples of lower weight (10 g).

The samples were cleaned by anodic etching for 3 minutes in a (1 + 7) mixture of sulphuric acid, sp.gr. 1·84, and methanol, a nickel cathode and a current of 1·5 amps being used. The samples were washed with three successive portions of methanol, dried with paper tissue, weighed and loaded into the apparatus as rapidly as possible. The samples were pre-heated in batches of five for 15 minutes at 1000° C.

Molybdenum samples were fused under two different sets of conditions; in an iron - tin bath at 1750° to 1800° C or directly in the graphite crucible at 2200° C.

The iron - tin bath was prepared initially by placing 20 g of vacuum-melted steel and 0·1 g of tin in the crucible; when this had been de-gassed and the furnace blank rate had fallen to 1 μl per minute, usually after 5 minutes, a sample of molybdenum was added. Before each subsequent addition of molybdenum, a further 10 g of steel and 0·1 g of tin were added to the contents of the bath. It was found essential to add the samples of molybdenum within 15 minutes of adding the steel. If the interval was more prolonged the bath became viscous and the evolution of gas from the molybdenum was slow and often incomplete. By using this technique a maximum of nine 10-g samples could be analysed per run.

The direct-melting technique was comparatively simple. Samples of molybdenum were added to the contents of the crucible at 2200° C, and the gas was collected for 1 minute, measured and analysed. The samples melted rapidly under these conditions owing, possibly, to the formation of a carbon - molybdenum eutectic. By this technique a maximum of twenty 10-g samples could be analysed during one run. This method was preferred to the iron-bath technique since it was simpler, more rapid and more reliable, particularly on samples having a low gas content. After additions of sample the furnace blank rate rapidly returned to its previous level. During the run the blank rate tended to decrease slightly whereas with the iron-bath technique it usually tended to increase slightly.

PROCEDURE FOR SILICON—

The samples of silicon for analysis were taken from vacuum- and argon-grown single crystals of approximate diameter 1 cm. These had been cut into slices 3 mm thick and optically polished for infrared examination; the slices had then been cut diametrically, and one half of each (weight 0·4 to 0·5 g) was used for the analysis.

The samples were weighed and then cleaned by etching for 15 seconds in a 10-ml polythene beaker with 5 ml of a (1 + 1) mixture of nitric acid, sp.gr. 1·42, and hydrofluoric acid, sp.gr. 1·15. The beaker rested in a large 8-inch diameter evaporating basin, and when etching was complete the beaker and contents were swamped with 200 ml of de-ionised water. The silicon was removed with platinum-tipped forceps, washed in fresh de-ionised water and then transferred to the vacuum lock as quickly as possible. Most of the adhering water was shaken off and the rest was removed in the first vacuum stage of the lock. It was found by experiment that 35 mg of sample were removed during the etching process, and, therefore, this amount was subtracted from the recorded weight. When in the vacuum system, the samples were pre-heated at 1000° C for 5 minutes before analysis.

An iron bath was used for the analysis since molten silicon attacks carbon rather vigorously. The crucible was maintained at 1700° C, which gave a furnace blank rate 0·3 to 0·5 μl per minute. Vacuum-melted steel (10 g) and 0·1 g of tin were used for the initial bath, and after every three analyses a further 10 g of steel and 0·1 g of tin were added.

PROCEDURE FOR OTHER METALS AND POWDERS—

The apparatus was also used successfully for samples of thorium in an iron bath at 1950° C and for chromium and copper at 1700° C. Samples of iron, ferro-silicon, ferro-molybdenum and ferro-aluminium powders were also analysed by wrapping them in nickel foil and fusing in an iron bath.

RESULTS

The mean results and standard deviations obtained on a series of typical vacuum-melted steels are shown in Table IV; the corresponding results obtained on sintered, arc-cast and zone-refined molybdenum are shown in Table V. Results obtained on two samples of single-crystal silicon grown under a partial pressure of argon (sample A) and in vacuum (sample B) are shown in Table VI.

TABLE IV

RESULTS OBTAINED ON VACUUM-MELTED STEELS

Sample number	Approximate weight of sample, g	Number of determinations	Mean, p.p.m.	Standard deviation, p.p.m.	Coefficient of variation, %
A379*	1·5	11	115	2·7	2·3
A329†	1·5	12	12·0	0·59	4·9
A437(b)‡.. ..	1·5	12	6·37	0·40	6·3
	10·0	6	6·35	0·33	5·2

* Vacuum-melted Swedish iron, containing less than 0·1 per cent. of total impurity.

† Vacuum-melted R.A.R.D.E. steel containing 1 per cent. of carbon, 1·8 per cent. of silicon, 1·4 per cent. of manganese, 1·3 per cent. of molybdenum, 0·6 per cent. of vanadium and 1·5 per cent. of copper.

‡ Vacuum-melted steel containing 0·6 per cent. of carbon with less than 0·1 per cent. of minor constituents.

TABLE V

RESULTS OBTAINED ON DIFFERENT TYPES OF MOLYBDENUM

Type of molybdenum	Approximate weight of sample, g	Conditions of fusion	Number of determinations	Temperature, °C	Mean oxygen content, p.p.m.	Standard deivation (1σ), p.p.m.	Coefficient of variation, %
Sintered bar X..	14	Iron - tin bath	8	1750	57·0	1·3	2·3
	14	No bath	7	2200	57·4	1·4	2·4
	2	No bath	13	2200	54·9	1·7	3·1
Sintered bar Y..	13	No bath	11	2200	42·5	0·8	1·9
	2	No bath	11	2200	42·8	1·1	2·5
Arc cast.. ..	10	Iron - tin bath	20	1750	1·32	0·40	30·0
	10	No bath	12	2200	1·97	0·16	7·9
	1·5	No bath	6	2200	2·66	0·63	24·0
Zone refined ..	10	No bath	4	2200	0·35	0·07	20·0
	1·5	No bath	4	2200	0·85	0·16	19·0

There was a significant difference between the means of the results on the large and small samples for the first sintered molybdenum bar (X) in which the samples were taken from different positions in the bar. A second bar (Y) was therefore analysed at different weights, and this time the large and small samples were taken from alternate positions along the bar; there was little difference in the means of these results.

With arc-cast ingots the mean value of the results obtained on the 10-g samples by using an iron bath was lower than that obtained by direct melting. The spread of the results obtained by the former method was 0·76 to 2·40 p.p.m. compared with 1·73 to 2·31 p.p.m. obtained by direct melting. In fact, the higher results on the iron - tin bath series corresponded to the results obtained by the direct-melting technique, which suggests that, for some samples, alloying with the bath metal was slow and evolution of gas incomplete. More consistent results might have been obtained with a higher ratio of iron to molybdenum in the bath, but this would have limited the number of determinations per run still further. The advantages of the direct-melting technique were thus apparent as twenty samples could be analysed per run with greater precision.

With zone-refined samples, there was a difference of 0·5 p.p.m. between results at different sample weights, which can be explained as being due to residual surface contamination. The results agree at both weights if a surface contamination of 0·8 μg per sq. cm is assumed.

The small samples were discs 8 mm in diameter and 2 mm thick with a total surface area of 1·5 sq. cm. A correction of 0·8 p.p.m. could be applied to the results for the small samples and 0·4 p.p.m. to those for the large samples (surface area 5·0 sq. cm). When these corrections were applied, the figure suggested that the oxygen content of the zone-refined molybdenum was less than 0·2 p.p.m.

TABLE VI

RESULTS OBTAINED ON 2 TYPES OF SINGLE-CRYSTAL SILICON

Type of single-crystal silicon	Number of determinations	Mean oxygen content, p.p.m.	Standard deviation (1σ), p.p.m.	Coefficient of variation, %
Argon grown	6	12·3	1·6	13
Vacuum grown	8	17·1	1·4	8·3

The ratio of 1·39 obtained on the results of the samples of silicon analysed by vacuum fusion compares favourably with the 1·47 from infrared measurements. Closer agreement would probably be obtained if the surface contamination of the silicon was determined and the results were corrected accordingly.

CONCLUSIONS

The vacuum-fusion technique has been developed successfully and applied to the analysis of steel, molybdenum and silicon at low levels of oxygen (less than 1 p.p.m. in molybdenum).

With samples of steel the coefficients of variation obtained were much less than 10 per cent., and no bias due to remaining oxide films was observed. There was good indication that the method was accurate down to 6 p.p.m., the lowest level encountered.

Coefficients of variation of less than 10 per cent. were obtained on the determination of oxygen in molybdenum down to 2 p.p.m., but at this lowest level there was a positive bias due to residual surface contamination. Analysis of zone-refined material suggested that most of the oxygen evolved came from the residual surface contamination and that the oxygen content of the material was in the range 0 to 0·2 p.p.m.

Analysis of 0·5-g samples of single-crystal silicon gave a coefficient of variation of 10 per cent. at the level of 17 p.p.m. of oxygen. The ratio between the levels of oxygen obtained on the two different series of samples agrees fairly well with the same ratio obtained by infrared examination at 9 μ.

We acknowledge the work done by the late Mr. A. Bacon who made a significant contribution to the progress of this work and thank Mr. R. J. Loneragan who carried out the infrared analyses and was responsible for some early work in the field.[25] Acknowledgment is also made to Mr. B. R. Watson-Adams, who carried out many of the analyses, for helpful suggestions and practical assistance.

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The Quantitative Determination of Active Hydrogen by a Modification of the Zerewitinoff Method

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The gasometric method for determining active hydrogen in organic compounds by using methyl magnesium iodide has been modified and made more accurate and precise. The sources and magnitudes of the errors in the determination are considered, and a method of correction is given. The method was developed for determining hydroxyl groups in silanols, but has been found applicable to a range of compounds containing active hydrogen. The effects of solvents and of impurities associated with silanols have also been studied. The accuracy of the method has been shown to be ±1·5 per cent. of the theoretical value, and it is possible to determine –OH percentages down to 0·04 per cent.

A METHOD for determining the hydroxyl content of silanols was required, which could be used for the routine control of the quality of silicone intermediates and resin solutions. Titration of the silanol group with Karl Fischer reagent has been studied by Gilman and Miller[1] and Grubb,[2] but our preliminary investigation of this method showed it to be of limited application, and it was decided to investigate the reaction between Grignard reagent and silanols.

The use of Grignard reagents for the quantitative determination of active hydrogen was first suggested by Tschugaeff,[3] and it was extensively studied by Zerewitinoff[4] and later modified by Roth.[5] Sudborough and Hibbert,[6] in studying the determination of amines by the Zerewitinoff technique, found that the methane generated during reaction was partly soluble in the reaction mixture, and they devised a method of correcting for this effect. Hibbert[7] apparently overlooked methane solubility as a cause of the low results obtained on the lower aliphatic alcohols, and, instead, he suggested that an equilibrium was attained. The introduction by Kohler and Richtmeyer[8] of an apparatus, in which the Grignard reagent was contained under an inert atmosphere of dry nitrogen, was an important development, and the first widely adopted procedure was that of Soltys,[9] who modified the Kohler apparatus. Hollyday and Cottle,[10] working with the lower aliphatic alcohols and using a modified Kohler apparatus, concluded that close control of the relative concentrations of reactants was necessary for quantitative results. However, they overlooked methane solubility as a possible cause of low results.

Several workers[6,11,12,13,14] have pointed out that methane solubility can affect the result of a determination, and various correction techniques have been developed, the simplest of which is to use pure methane as the inert gas in the apparatus; this has been successfully adopted by Lehmann and Basch,[11] Brown and Hafliger[12] and Guenther.[13] Villars[14] derived a factor that, when multiplied by the observed volume of methane, corrected for methane solubility; however, no mention was made of a correction for the vapour pressure effect of the reaction mixture. Many methods[15,16,17] involving the use of special apparatus have been developed, which give reasonable results without the necessity of correcting for methane solubility; but it was felt that none of these methods was suitable for routine use. The choice of solvent for the sample is important and has often been stressed.[4,8,10,18] The formation of insoluble products usually leads to low results,[19] presumably because of occlusion of unreacted sample. Heating the reaction mixture has, in some instances, increased the result.[4,20] Constant temperature control of the gases inside the apparatus during determinations is extremely important, and several papers refer to this point,[10,11,13,16] although few have rigidly applied it in practice.

In a review, Olleman[21] states "Although several investigators have reported results within 1 to 2 per cent. of the theoretical values, in general reproducibility and accuracy are probably ±3 to 5 per cent."

As a result of our study of the literature, preliminary experiments were carried out, and the conclusions listed below were reached—

(*a*) The solvent for the sample should be easily dried and purified, possess a relatively low vapour pressure at the temperature of the determinations, and be completely miscible with Grignard reagent. Xylene fulfils these requirements.

(*b*) The use of pure methane as the inert gas in the apparatus is to be preferred, since it will completely eliminate errors due to methane solubility, because both the reagent and the solvent for the sample can be saturated with methane before reaction. Unfortunately, there is no ready supply of methane of 99·9 per cent. purity in this country. Nitrogen was chosen as the inert gas because a pure grade is readily available, and it has been extensively used by previous workers. However, its use does introduce the need for solubility corrections, which are discussed below.

(*c*) The methane generated by the sample on reaction with the Grignard reagent is partly soluble in the reaction mixture, the exact volume dissolved depending on the partial pressure of the methane and on the temperature.

(*d*) The experimental procedure for a determination is such that, before reaction, both the solvent for the sample and the Grignard reagent are saturated with nitrogen. After reaction, because of the methane generated, there is a decrease in the partial pressure of the nitrogen and some of the dissolved nitrogen is liberated from the reaction mixture.

(*e*) Because of the solubility effects discussed in (*c*) and (*d*), the volume of solvent for the sample, the volume of Grignard reagent added and the volume of nitrogen in the system before reaction must be kept constant for all determinations.

(*f*) The blank value, which is the increase in volume observed on mixing solvent and Grignard reagent alone, is the sum of two values: (*i*) the volume of methane generated by traces of active hydrogen (if any) in the solvent, and (*ii*) the volume of vapour produced by the reaction mixture in the presence of a constant volume of nitrogen.

(*g*) After reaction of a sample the total volume of the system increases, and there is a corresponding increase in the volume of vapour produced by the reaction mixture.

(*h*) The volume of the system and all the effects discussed above are temperature dependent, and the temperature of the system must be accurately controlled. A working temperature of 25° ± 0·1° C was chosen as convenient.

Hence, corrections must be applied for the solubility of the methane, the change in solubility of the nitrogen and the volume change due to the vapour pressure of the reaction mixture.

An equation was derived that related the theoretical volume of methane to the observed volume, the blank value and the correction terms for the solubility and vapour pressure effects referred to above. However, to obtain values for these correction terms, the coefficients of solubility of pure methane and of nitrogen in the reaction mixture and also the vapour pressure of the reaction mixture were required. Since these values were unknown the equation was simplified and the correction terms were represented by a single term, V_c, as shown in the equation below.

$$V_t - (V_o - B) = V_c \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where V_t = the theoretical volume of methane generated by the sample,
V_o = the observed volume increase after reaction and
B = the blank volume.

(Equation 1 applies to a system at constant temperature and pressure and containing a constant volume of nitrogen gas and a reaction mixture of constant volume and composition.)

For a known weight of a pure standard substance, if the reaction is assumed to be stoicheiometric, V_t can be calculated; from the value of $(V_o - B)$, obtained experimentally, the corresponding value of the correction V_c can be calculated by using equation 1. Thus by varying the weight of standard substance a series of values of $(V_o - B)$ and V_c can be obtained and a graph plotted of $(V_o - B)$ against V_c.

Hence, from the value of $(V_o - B)$ obtained for a sample the corresponding correction V_c may be obtained from the graph. Then V_t for the sample is given by—

$$V_t = (V_o - B) + V_c \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

The experimental work described consisted in investigating the accuracy and precision of this proposed graphical correction procedure for several silanols. It was extended to include the determination of active hydrogen in alcohols, phenols, carboxylic acids and amines, and the effects of solvents and siloxanes on the method were studied.

Because water is formed by the condensation of silanols to siloxanes, there is a possibility that water may be present as an impurity in silanol samples, either in solution or as free water droplets. Hamelin[22] suggests that water reacts quantitatively with Grignard reagent, and so the effects of water both as a dilute solution in toluene and as free water droplets were examined. The solution of water in toluene was prepared by shaking together water and toluene at a temperature below room temperature, and, after separation, the toluene was decanted off. This solution remained clear and bright at room temperature, and the water concentration was found by titrating with Karl Fischer reagent.

β-Naphthol was chosen as the standard substance for the preparation of the calibration graph because it is readily available, easily purified by recrystallisation and stable if stored in a dark bottle. A working temperature of 25° ± 0·1° C was used, and all volumes were corrected to standard pressure.

METHOD

REAGENTS—

Magnesium metal, granular—Dry laboratory-reagent grade magnesium for 3 hours in an oven at 135° C immediately before use.

n-Dibutyl ether—Boil under reflux with sodium metal for 1 hour; then distil, and reject the first 50 ml of distillate.

Methyl iodide—Laboratory-reagent grade is satisfactory.

Nitrogen, dry—Pass nitrogen from the cylinder over a mercury safety bottle into a column containing soda lime, to remove traces of carbon dioxide, and then through activated alumina and molecular sieves to remove water. Finally, pass the nitrogen through a 125-ml Dreschel bottle, containing silicone fluid MS200/350cS to a depth of about 6 cm, so that the bubble rate can be observed and controlled.

Solvent xylene—Boil xylene under reflux for 1 hour with sodium metal, and then distil. Reject the first 50 ml of distillate, but collect a suitable fraction in a clean dry bottle. The dry xylene from this bottle should be transferred as required into a 10-oz bottle fitted with a glass stopper. To this xylene add small pieces of sodium metal, set aside until effervescence ceases, and then stopper securely. Always replace the stopper tightly after use.

Grignard reagent—Prepare a 0·75 M solution of methyl magnesium iodide in n-dibutyl ether.[17] This solution, when stored under nitrogen in a well-stoppered bottle, is stable for several months.

APPARATUS—

Constant temperature water-bath and external circulating pump—A constant temperature water-bath capable of controlling the temperature of the water passing through the apparatus at 25° ± 0·1° C is required. A suitable water-bath is manufactured by Townson and Mercer, catalogue number E.270. Water from the bath is forced through the apparatus by a pump capable of supporting a water-head of about 4 feet. Water passes through the circuit in the direction shown in Fig. 1 and overflows from the side-arm of the beaker back to the water-bath.

Reaction flasks—Flat-bottomed 25-ml flasks (tolerance ±2 ml) fitted with B19 ground-glass joints and glass lugs. Dry in an oven at 135° C for at least 1 hour before use, and cool in a desiccator.

Syringe—All-glass 2-ml syringe fitted with a No. 17 stainless-steel hypodermic needle. When not in use, store the syringe in a desiccator.

Reaction apparatus—This is essentially that described by Soltys.[9] Assemble as shown in Fig. 1, in such a way that it is pivoted to allow the contents of the reaction flask to be shaken during a determination. Thoroughly dry the apparatus in an oven at 150° C before assembling, and flush with dry nitrogen at a flow rate of 3 bubbles per second for half an hour, with a dry flask attached and the taps in position A (see Fig. 2). Do not stopper the reservoir. When the apparatus has been flushed, turn the taps to position B (see Fig. 2), and fill the reservoir two-thirds full with Grignard reagent; clean and grease the joint with Vaseline, and stopper securely.

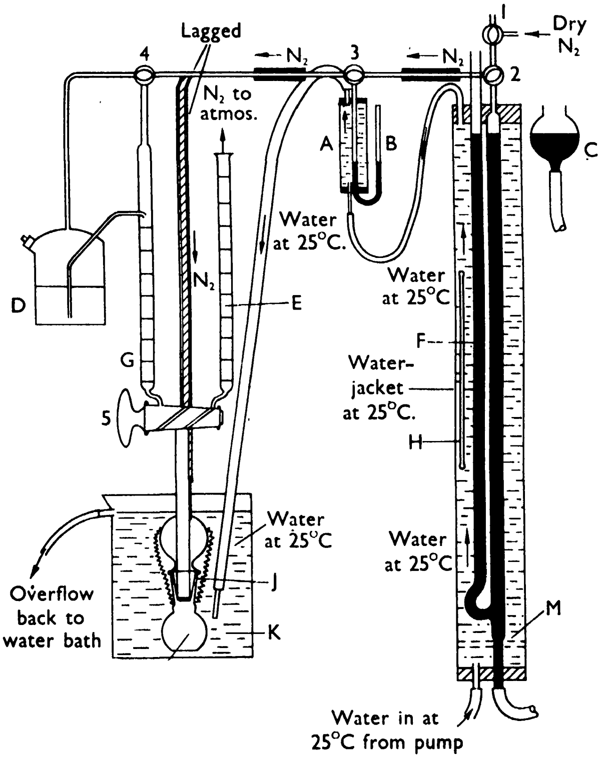
DETERMINATION OF THE BLANK VALUE—

All tap positions referred to in the method are shown in Fig. 2.

With the syringe place 2 ml of the dry xylene in a reaction flask, taking care not to include any sediment from the xylene bottle. Grease the upper half of the B19 cone joint with Vaseline, and attach the flask, securing it by means of springs. Totally immerse the flask and the glass bulb above the joint in the water, at 25° ± 0·1° C, contained in the special beaker. The water temperature in the jacket and beaker must be maintained at 25° ± 0·1° C throughout all determinations.

Turn taps 1, 2, 3, 4 and 5 (see Fig. 1) to position B, and flush the apparatus for 15 minutes with dry nitrogen. Then fill the reagent burette to the zero mark with Grignard reagent as described below.

Turn taps 1 to 5 to position C. In this position the nitrogen pressure builds up and excess of nitrogen vents off through the mercury safety bottle. Lower the mercury reservoir slowly to draw the reagent over into the burette. When the burette is full to the zero mark rapidly raise the mercury reservoir, and approximately equilibrate the mercury levels in the gas burette. This raising of the reservoir when the reagent reaches the zero mark in the reagent burette is necessary to prevent reagent being drawn into the connecting tubes between the apparatus and gas burette. With the mercury levels approximately equal in the gas burette, return the taps to position B for a few seconds. Now turn the taps, in numerical order, to position D.



A = Water-jacket
B = Dibutyl phthalate manometer
C = Mercury reservoir
D = Reagent reservoir
E = Side-arm
F = 50-ml gas burette

G = 10-ml reagent burette
H = Thermometer
J = B19 cone
K = Special beaker
L = Reaction flask
M = Cork (for support)

Fig. 1. Constant-temperature apparatus for determining active hydrogen by a modification of the Zerewitinoff method

Check that the system is gas-tight by lowering the mercury reservoir by about 20 cm. Set aside for 2 minutes, noting whether or not the mercury levels remain steady. If a leak is detected, raise the reservoir, clean and re-grease taps 1, 2, 3 and 4, and then repeat the procedure for testing for leaks. When the system is gas-tight, raise the mercury reservoir and turn the taps to position E. Adjust the level of the mercury in the gas burette, and note the reading.

N.B.—Always return the mercury to the same level in the burette before proceeding with a determination.

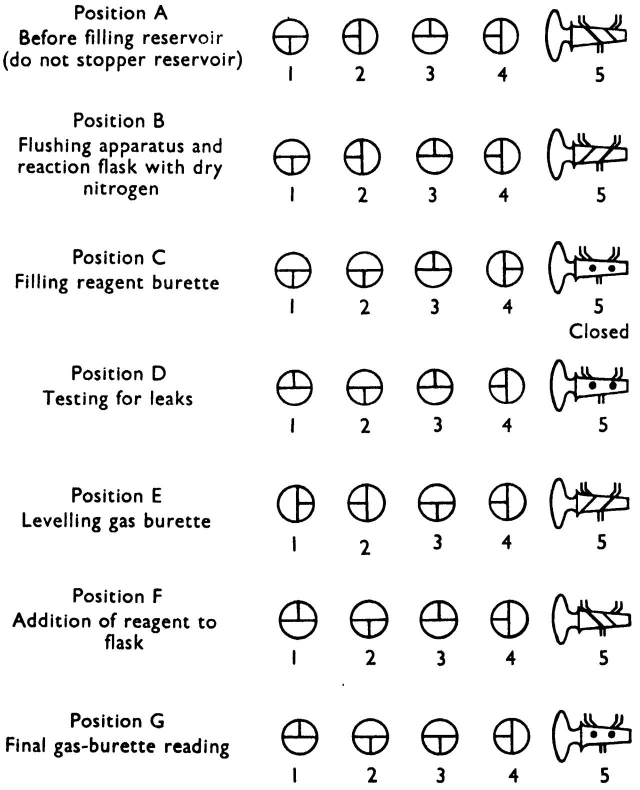


Fig. 2. Diagram of tap positions

Note the reading in the reagent burette, and return the taps to position D. Lower the mercury reservoir to create a small partial vacuum in the reaction flask, and while maintaining this small vacuum turn only tap 5 to position F until 5 ± 0·1 ml of reagent is drawn from the burette into the flask. Close tap 5, and approximately equilibrate the mercury levels in the gas burette. Shake the flask and contents for 2 minutes, keeping the mercury levels in the gas burette equal during the evolution of any gases. After the evolution of gas has ceased, set the flask aside for a further 3 minutes with occasional shaking. Adjust the mercury levels in the gas burette until they are approximately equal. Now turn tap 3 to position G thus bringing the dibutyl phthalate manometer into the system, and adjust the mercury reservoir so that the pressure in the system equals atmospheric pressure. Note the reading in the gas burette.

Calculate the volume occupied by the blank from the recorded increase in volume, after subtracting the volume of Grignard reagent added to the flask.

Check the blank value daily before proceeding with an analysis.

CLEANING THE APPARATUS—

Turn the taps to position E. Clean the flask, the joint and reagent delivery tube with dilute hydrochloric acid, water, acetone and finally with diethyl ether. Dry the flask in an oven, and wipe the inside and outside of the joint with a clean dry cloth. Attach an empty reaction flask to the joint, and flush with dry nitrogen for 3 minutes with the taps turned to position B.

COMPOUNDS ANALYSED—

Table I shows the purification procedure and constants of these compounds.

TABLE I

PURIFICATION PROCEDURES AND CONSTANTS OF COMPOUNDS ANALYSED

Compound	Melting-point Cited in literature, °C	 Found, °C	Notes
Tetramethyldisiloxane-1,3-diol	67 to 68[25]	68·5	Recrystallised twice from light petroleum
Diphenylsilanediol	—	165	—
Diphenyldimethyldisiloxane-1,3-diol	—	115·5	—
Triphenylsilanol	150·5 to 151·5[25]	151	Recrystallised twice from benzene
Tetraphenyldisiloxane-1,3-diol	108 to 109[13]	109	—
β-Naphthol	122[26]	122	AnalaR grade, recrystallised twice from carbon tetrachloride and stored in a dark bottle in a desiccator. Used to prepare calibration graph
α-Naphthol	96[26]	95	Recrystallised from carbon tetrachloride
Resorcinol	110[26]	110	Recrystallised twice from benzene and stored in a dark bottle
Phenol	41[26]	41	AnalaR grade
Thymol	51·5[26]	50·5	B.D.H. laboratory-reagent grade
Salicylaldehyde	196·5[26] (boiling-point)	196 (boiling-point)	G.P.R. grade, redistilled and mid-cut collected
Vanillin	82[26]	82	Micro-analytical-reagent grade
Benzoic acid	122[26]	122	Micro-analytical-reagent grade, ground to powder
Glacial acetic acid	—	—	AnalaR grade, 97·6 per cent. pure
n-Butanol	—	—	AnalaR grade
Octan-2-ol (capryl alcohol)	—	—	Micro-analytical-reagent grade
Aniline	184·4[26] (boiling-point)	184 (boiling-point)	AnalaR grade, freshly redistilled and mid-cut collected
Cyclohexylamine	134[26] (boiling-point)	134 (boiling-point)	Hopkin & Williams fine-chemicals grade, freshly redistilled and mid-cut collected
Di-n-propylamine	110·7[26] (boiling-point)	110 (boiling-point)	B.D.H. laboratory-reagent grade, freshly redistilled and mid-cut collected

PREPARATION OF THE CALIBRATION GRAPH—

NOTE—A calibration graph refers only to the apparatus with which it has been prepared. If the apparatus suffers a change in volume exceeding 5 ml, a new calibration graph must be prepared.

Into a clean dry flask weigh accurately between 0·03 and 0·19 g of β-naphthol, and with the syringe add 2 ± 0·1 ml of sodium-dried xylene. Attach the reaction flask to the apparatus, and proceed exactly as described for the blank determination.

Record the final total volume in the gas burette, and calculate the volume collected to the nearest 0·1 ml. Record the corrected barometric pressure at the time of the determination, *i.e.*, mm of mercury at 0° C.[23] Correct the volume collected to the standard pressure to obtain V_0; subtract the blank value to obtain $(V_0 - B)$.

Calculate the theoretical volume of methane evolved by the known weight of sample at standard pressure and 25° C, from the equation—

$$V_t = \frac{W}{144{\cdot}2} \times 24{,}470 \text{ ml}$$

where W = the weight of β-naphthol taken, in grams. Then the correction, V_c, in millilitres,

$$= V_t - (V_o - B).$$

Repeat the procedure on weights of β-naphthol, such that the range of gas volumes collected is between 5 and 30 ml. Plot a graph of V_c against $(V_o - B)$. A typical calibration graph is shown in Fig. 3.

SAMPLE ANALYSIS—

Weigh accurately into a clean dry flask a suitable amount of the sample to produce about 25 ml of methane. From the syringe carefully add 2 ± 0·1 ml of the dry xylene. Now proceed exactly as described for the blank determination. The time required for complete evolution of gas depends on the reactivity of the sample, but after the evolution of gas has ceased the flask must be set aside for a further 3 minutes, with occasional shaking, to attain equilibrium conditions before making the final adjustments to the manometer.

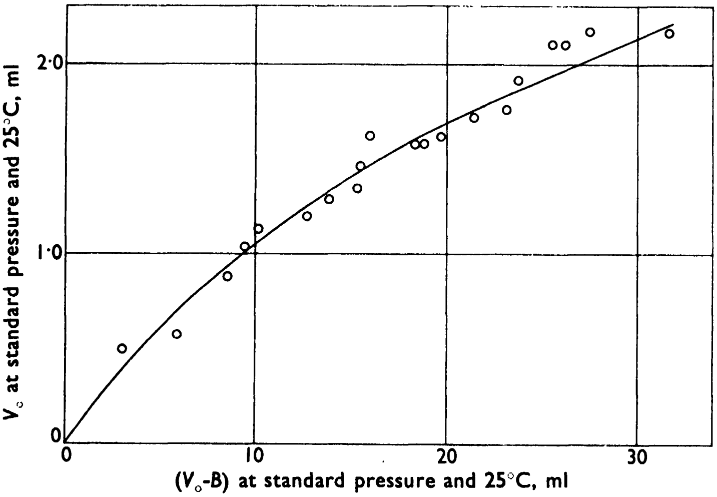


Fig. 3. Typical calibration graph

Correct the volume collected to standard pressure, subtract the blank volume, and by reference to the calibration graph read off the corresponding correction volume, V_c, to the nearest 0·1 ml.

ADD the correction to the value of $(V_o - B)$ to give the true volume, V_t, of methane evolved by the known weight of sample.

$$V_t, \text{ ml} = (V_o - B) + V_c, \text{ at S.P. and } 25^\circ \text{ C}.$$

Then

$$\text{-OH per cent. w/w} = \frac{V_t \times 1{\cdot}7}{\text{Sample weight} \times 24{\cdot}47}$$

DISCUSSION OF RESULTS

The statistical treatment of the results obtained on four pure silanols having -OH percentages from 6·1 to 20·5 per cent. are shown in Table II. No bias was found, and the application of Bartlett's variance ratio test[24] showed that the apparent difference in the variances is non-significant.

The results shown in Table III, for the analysis of another silanol and some alcohols, phenols, carboxylic acids and amines, indicate that the method is applicable to a wide range of compounds containing active hydrogen. The reaction time for the compounds listed was less than 5 minutes, with the exception of cyclohexylamine and di-n-propylamine. The low results obtained with vanillin and glacial acetic acid may be due to occlusion of unreacted sample in the precipitates that both substances formed on reacting with the Grignard reagent.

The results for the reaction of water in droplet form and in toluene solution were less than half the theoretical value, if it is assumed that there is only one active hydrogen atom per mole of water. When water droplets were present the reaction was slow and the droplets became coated with a white precipitate until reaction eventually ceased. Heating the reaction mixture during the determination had no effect on the result. Similarly, water in toluene solution did not react completely, and a faint white precipitate was observed each time.

Presumably these low results were caused either by occlusion of unreacted water by the precipitate or by the formation of hydrated magnesium salts. This problem has been discussed by Hamelin,[22] but our experimental results do not agree with his conclusions.

No significant effect was observed on the result of a standard β-naphthol analysis when amounts of up to 2 ml of dry xylene, toluene or benzene were added to the 2 ml of xylene used as solvent in the proposed method. Presumably, as the methane solubility increases, the volume of nitrogen liberated from the reaction mixture also increases, and the net volume change does not cause a significant error. However, the addition of 2 ml of light petroleum caused interference, and low results were obtained.

TABLE II

STATISTICAL TREATMENT OF THE RESULTS OBTAINED ON PURE SILANOLS

Compound	Number of determinations	Mean purity found, % w/w	Variance	Standard deviation
Tetramethyldisiloxane-1,3-diol	20	99·9	0·41	0·64
Diphenylsilanediol	20	100·0	0·58	0·76
Diphenyldimethyldisiloxane-1,3-diol	9	100·1	0·33	0·58
Triphenylsilanol	5	99·8	0·77	0·88

TABLE III

RESULTS OBTAINED FOR VARIOUS COMPOUNDS

Compound	Found, –OH % w/w	Mean of results	Theory, –OH % w/w
Tetraphenyldisiloxane-1,3-diol	8·3, 8·2	8·25	8·21
α-Naphthol	12·0, 11·9	12·0	11·8
Resorcinol	31·0, 30·6	30·8	30·88
Phenol	18·3, 18·3	18·3	18·1
Thymol	11·4, 11·4	11·4	11·32
Salicylaldehyde	13·9, 13·8	13·9	13·92
Vanillin*	4·8, 7·4, 7·7†	—	11·17
Benzoic acid	13·9, 14·2, 14·0, 14·2	14·0	13·93
Glacial acetic acid*	26·7, 26·9	26·8	29·82
n-Butanol	22·9, 22·6, 23·2	22·9	23·07
Octan-2-ol (capryl alcohol)	13·1, 13·1, 13·0	13·1	13·06
Aniline	16·2, 16·1‡	16·2	16·12
Cyclohexylamine§	15·5, 15·3	15·4	15·14
Di-n-propylamine‖	14·8, 14·7	14·8	14·83

* Precipitate formed on reaction.
† After heating reaction mixture at 100° C for 5 minutes.
‡ Results expressed as –NH per cent. w/w.
§ Reaction time 15 minutes.
‖ Reaction time 60 minutes.

Low results were also obtained on standard β-naphthol when small amounts of acetone were added, but in this instance an insoluble product was formed between the acetone and the Grignard reagent.

Because silanols can contain various siloxanes as impurities, mixtures of equal weights of siloxane and standard β-naphthol were analysed to ascertain whether or not the siloxanes interfered in the determination. No interference was observed from—(*a*) hexamethylcyclotrisiloxane, (*b*) octamethylcyclotetrasiloxane, (*c*) decamethylcyclopentasiloxane, (*d*) a mixture of dimethylcyclopolysiloxanes, (*e*) linear dimethylpolysiloxanes trimethylsilyl endblocked

(MS 200 fluids), (*f*) a mixture of phenylmethylcyclopolysiloxanes, (*g*) 2,4,6,8-tetramethylcyclotetrasiloxane or (*h*) a mixture of linear methylhydrogenpolysiloxanes trimethylsilyl endblocked (MS 1107 fluid).

CONCLUSIONS

The method is applicable to a wide range of compounds, and, with the exceptions of acetone and light petroleum, no interference with the substances tested has been encountered.

A low result is obtained if a precipitate is formed when the substance reacts with Grignard reagent. For samples containing water, it is essential that the water be removed before determining active hydrogen, because water does react with Grignard reagent although the reaction is not quantitative.

The method has been improved, and the accuracy of the graphical correction procedure is ± 1·5 per cent. of the theoretical value. It is possible to determine -OH percentages down to 0·04 per cent.

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The Micro-determination of Sulphur in Organic Compounds by Fusion with Sodium Peroxide and Titration of the Sulphate with Barium Perchlorate

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The method described by Inglis for the volumetric micro-determination of sulphur in organic compounds has been critically examined. The method involves fusion of the sample with sodium peroxide in a metal bomb, removal of sodium from the aqueous extract of the fusion product by means of a cation-exchange resin and titration of the sulphate ion with barium perchlorate. Each of these operations has been investigated, and modifications have been introduced to improve the method. This modified procedure is described, and results are given to illustrate the accuracy and precision achieved in the analysis of several organic sulphur compounds, including some containing nitrogen, chlorine or fluorine as additional elements.

ALTHOUGH the oxygen-flask combustion technique[1] combined with the barium perchlorate titration procedure[2] is a well established method for determining sulphur in organic compounds, it is necessary on occasion to use an alternative technique, such as fusion with sodium peroxide in a micro-bomb, for the decomposition of the sample. A serious disadvantage of this fusion procedure is that the large amount of sodium in the extract of the fusion product interferes in the subsequent titration with barium perchlorate. In an attractive method devised by Inglis[3] this defect is overcome by the use of a cation-exchange resin for the removal of sodium. Investigations carried out in this laboratory have confirmed that the method is basically satisfactory, and have suggested some modifications for the improvement of its convenience and reliability.

EXPERIMENTAL

TITRATION OF SULPHURIC ACID WITH BARIUM PERCHLORATE—

Since we were interested in the analysis of organic sulphur compounds containing chlorine, fluorine or nitrogen, the effect of these elements on the titration of sulphuric acid was examined. A series of equal portions of 0·025 N sulphuric acid, each corresponding to 0·875 mg of sulphur, were measured in a content pipette and diluted to 10 ml. Each solution was titrated with aqueous 0·01 N barium perchlorate after the addition of 70 ml of ethanol, 0·1 ml of 0·5 per cent. thorin solution, 0·1 ml of 0·03 per cent. methylene blue solution and different amounts of hydrochloric acid, hydrofluoric acid or nitric acid. The solutions were stirred magnetically throughout the titrations, and the appearance of a pink colour, persisting for at least 20 seconds, was taken as the end-point. In these and all subsequent titrations the mixed indicator recommended by Wagner[4] was used in preference to thorin alone, and, for reasons given elsewhere,[5] the aqueous barium perchlorate was preferred to the alcoholic solution proposed by Fritz and Yamamura.[2] The results obtained in this series of experiments (see Table I) show that, when an error of about 1 per cent. is acceptable, amounts of chlorine, fluorine or nitrate up to about 2 mg can be tolerated, and that the effect of larger amounts of fluorine can be suppressed by adding boric acid.[6] Some impairment of the end-point was observed if the amount of boric acid used was much in excess of 30 mg.

Two precautions taken in the use of barium perchlorate may be mentioned here. Standardisation of the titrant and all subsequent titrations of sample solutions were always carried out by the same operator, and care was taken to ensure that the correct proportion of methylene blue to thorin was used. The significance of the second precaution was established by the result of an experiment in which six equal volumes of 0·025 N sulphuric acid, each corresponding to 0·875 mg of sulphur, were measured in a content pipette and diluted to 25 ml.

Each solution was then titrated with barium perchlorate after the addition of 100 ml of ethanol, 0·1 ml of 0·5 per cent. thorin solution and different amounts of 0·03 per cent. methylene blue solution. Satisfactory end-points were obtained with 0·1 to 0·14 ml of methylene blue, but the titration values tended to increase over the range. With still larger volumes of methylene blue, the titration values continued to rise and the end-points became difficult or even impossible to detect. The results are shown in Table II.

TABLE I

TITRATION OF SULPHURIC ACID (≡ 0·875 mg OF SULPHUR) IN THE PRESENCE OF CHLORIDE, FLUORIDE OR NITRATE

Weight of additional element, mg	Sulphur found in presence of chloride, mg	fluoride, mg	nitrate, mg
0·0	0·878	0·877	0·873
0·5	0·881	- -	0·876
1·0	0·881	0·879	0·880
2·0	0·885	0·884	0·881
3·0	0·887	0·902	0·886
4·0	0·888	0·962	—
4·0 (+ boric acid)	—	0·875	

TABLE II

TITRATION OF SULPHURIC ACID (≡ 0·875 mg OF SULPHUR) IN THE PRESENCE OF DIFFERENT AMOUNTS OF 0·03 per cent. METHYLENE BLUE SOLUTION

Volume of methylene blue solution, ml	Sulphur found, mg
0·1	0·876
0·12	0·879
0·14	0·886
0·16	0·891
0·18	0·908
0·20	> 0·908

REMOVAL OF SODIUM BY MEANS OF CATION-EXCHANGE RESIN—

In the procedure described by Inglis[3] the aqueous extract of the sodium peroxide fusion product is diluted to 50 ml, and about 15 ml of this solution was used to rinse about 30 g of moist cation-exchange resin. The remainder of the solution is then stirred with the resin for several minutes, and decanted. A 25-ml portion of the solution is titrated with alcoholic barium perchlorate after the addition of 100 ml of ethanol and a few drops of 0·2 per cent. thorin indicator solution.

In order to conserve the sample solution an attempt was made to modify this procedure in a manner such that a 25-ml portion of the solution could be added directly to the moist resin and the resulting sulphuric acid quantitatively recovered for titration. The recovery of the sulphuric acid by rinsing the resin with water was not investigated, since the final volume of solution to be titrated after the addition of the appropriate amount of ethanol would have been excessive. Satisfactory recovery of sulphuric acid was obtained by the use of ethanol in a series of experiments with solutions of sodium sulphate containing the equivalent of 0·999 mg of sulphur per 25 ml. For these tests a quantity of Amberlite IR-120(H) was washed with hydrochloric acid, water, ethanol, water, sodium hydroxide and water, in that order, as described under "Method," p. 795. About 30 g of the moist resin were shaken in a stoppered conical flask with about 20 ml of ethanol to remove some of the water. The solution was decanted, and the resin shaken for 5 minutes with 25 ml of the sodium sulphate solution. This solution was then decanted, and the resin washed with four successive 25-ml portions of ethanol. The combined solutions were titrated with barium perchlorate in the usual manner. Since this procedure was required for use with the aqueous extracts of the sodium peroxide fusion products of organic sulphur compounds, these tests were repeated with a series of solutions each containing 0·4 g of sodium hydroxide in addition to sodium sulphate equivalent to 1·012 mg of sulphur. The amount of sodium hydroxide was chosen to approximate to the amount expected in the extracts of sodium peroxide fusion products. The satisfactory results obtained in both series of tests are shown in Table III.

TABLE III

DETERMINATION OF SULPHUR IN SODIUM SULPHATE SOLUTIONS AFTER REMOVAL OF SODIUM BY MEANS OF A CATION-EXCHANGE RESIN

Test solution	Sulphur found, mg	Mean, mg	Precision (standard deviation)
Sodium sulphate 0·999 mg of sulphur)	1·00, 1·01, 1·00, 1·01, 1·01, 1·01, 1·02, 1·02, 1·01, 1·01, 1·00, 1·01	1·01	±0·007
Sodium sulphate 1·012 mg of sulphur) +0·4 g of sodium hydroxide	1·01, 1·02, 1·02, 1·01, 1·01, 1·01, 1·03, 1·02, 1·03, 1·00, 1·04, 1·02, 1·02, 1·02	1·02	±0·01

At this stage of the work the exchange capacity of the resin was determined on two 32-g portions of moist resin containing about 31 per cent. of water, as determined by the loss in weight of a separate portion of resin after it had been dried in air for about 4 hours. One portion was washed with ethanol and then shaken for 5 minutes with successive 25-ml portions of 0·5 N sodium hydroxide. After each addition the resin was washed with ethanol. Exhaustion of the resin, as shown by the presence of alkali in the decanted solution, occurred with the seventh portion of test solution. The weight of sodium quantitatively exchanged was therefore about 1·7 g. The second portion of resin was first washed with water and then shaken with successive 25-ml portions of the test solution as before, except that the resin was washed with water in place of ethanol after each addition of the test solution. The weight of sodium exchanged before exhaustion of the resin was again about 1·7 g.

DECOMPOSITION OF THE SAMPLE BY FUSION WITH SODIUM PEROXIDE—

In the interests of speed and convenience, an attempt was first made to carry out the sodium peroxide fusion procedure in an electrically fired stainless-steel micro-bomb[7] instead of in the gas-heated nickel bomb used by Inglis.[3] The organic sample, mixed with about 1 g of sodium peroxide and 60 mg of starch, was decomposed in the electrically fired bomb, and the aqueous extract of the fusion product was diluted to 50 ml. A 25-ml aliquot of this solution was shaken with cation-exchange resin, and the decanted solution titrated with barium perchlorate in the presence of ethanol. The results obtained with sulphonal (sulphur calculated = 28·09 per cent.) ranged from 21·0 to 27·8 per cent. of sulphur, and the titration end-points were unsatisfactory.

In a further series of analyses with benzyl disulphide (sulphur calculated = 26·03 per cent.) as the test substance, the results ranged from 25·2 to 25·9 per cent. of sulphur, the end-points were again unsatisfactory, and no improvement was effected by the use of glycol[3] in place of starch. An attempt was next made to carry out the fusion procedure in a gas-heated Parr micro-bomb constructed from the alloy "Ilium," but the results obtained were erratic and the titration end-points abnormal. The cause of the unsatisfactory end-points was subsequently traced to the presence of chromium in the fusion products. The chromium derived from the stainless-steel bomb and from the Ilium alloy was present as chromate in the extracts of the fusion products. In this form, chromium is not removed by a cation-exchange resin, and is known to interfere in the barium perchlorate titration. In addition to this source of error, the extremely low values obtained with the electrically fired bomb were probably caused by incomplete decomposition of the sample during the very short fusion period. Much improved results were obtained in a series of analyses in which the samples were fused with sodium peroxide and glycol in a nickel bomb as described by Inglis,[3] but occasional unsatisfactory values suggested the need for closer control of the fusion process.

In a series of experiments carried out with a nickel bomb of the type designed for use in the potassium fusion of fluorine compounds,[8] the samples were heated with sodium peroxide and glycol at various temperatures and for different times. Excellent results were obtained by heating for 2 to 3 minutes at 650° C in a muffle furnace. In all tests the fusion product extracts contained only small amounts of insoluble matter, but if heating was prolonged to above about 3 minutes considerable amounts of insoluble matter were produced by chemical attack on the bomb. Since the addition of glycol to the sodium peroxide was thought to be unnecessary, a further series of tests was carried out with sodium peroxide alone. In these tests the samples were mixed with about 1 g of sodium peroxide and heated for 2·5 minutes at 650° C. The fusion products were extracted with water, the extracts diluted to 50 ml, and a 25-ml aliquot was treated with cation-exchange resin before titration with barium perchlorate. The results obtained with a variety of sulphur compounds, including some containing nitrogen, fluorine or chlorine as additional elements, are shown in Table IV.

TABLE IV

DETERMINATION OF SULPHUR IN VARIOUS COMPOUNDS

Compound	Sulphur present, %	Sulphur found, %	Mean, %	Precision (standard deviation)
Benzyl disulphide	26·03	26·05, 26·1 26·10, 26·15 26·05, 25·80 25·80, 25·75 26·10, 26·20 25·70, 26·20	26·0	±0·18
Sulphonal	28·08	28·30, 28·20 28·30	28·27	—
Sulphanilic acid	18·51	18·40, 18·70 18·60, 18·40	18·52	—
Sodium dodecylsulphate	11·09	11·10, 11·20 11·00	11·10	--
S-Benzyl thiuronium chloride	15·80	15·90, 15·80 15·70, 16·00 15·70, 15·80 15·90, 15·90 15·70, 15·70	15·81	±0·11
Benzyl disulphide *m*-dinitrobenzene	26·03	26·20, 26·10 26·10, 26·20 25·90, 26·10 26·20, 26·20 25·90, 26·20 25·90, 26·10	26·10	±0·12
Benzyl disulphide + fluorobenzoic acid	26·03	26·00, 25·80 26·10	25·97	—

TABLE V

FORMATION OF NITRATE BY FUSION OF NITROGENOUS ORGANIC COMPOUNDS WITH SODIUM PEROXIDE

Compound	Weight taken, mg	Nitrogen present, mg	Nitrogen, expressed as nitrate, mg	Nitrate found, mg
S-Benzyl thiuronium chloride	10·158	1·400	6·197	0·275
	10·026	1·385	6·138	0·285
	9·650	1·333	5·900	0·270
Benzyl disulphide + *m*-dinitrobenzene	7·480 + 8·436	1·406	6·224	0·500
	7·360 + 8·330	1·389	6·148	0·540

In the analyses of mixtures of benzyl disulphide and fluorobenzoic acid the amounts of the fluorine compound present were in each test equivalent to about 2 mg of fluorine. Interference by fluoride ion was suppressed by the addition of about 30 mg of boric acid to the solutions immediately before titration. This procedure was preferred to the addition of a controlled amount of sodium hydroxide recommended by Inglis.[3] Before the analysis of the nitrogenous sulphur compounds was attempted, the amount of nitrate produced by the fusion of such compounds with sodium peroxide was determined, *S*-benzyl thiuronium chloride and a mixture of benzyl disulphide with *m*-dinitrobenzene being used as the test substances. The weighed samples were fused with sodium peroxide at 650° C in a nickel bomb, and the aqueous extracts of the fusion products were diluted to 50 ml in each test. The nitrate contents of the solutions were then determined by a colorimetric method; the results are shown in Table V.

The amounts of nitrate found in all these tests were less than the amount (2·0 mg) known to interfere significantly in the barium perchlorate titration. With *S*-benzyl thiuronium chloride the conversion of nitrogen to nitrate was about 4·5 per cent. compared with about 8·5 per cent. for the mixtures of benzyl disulphide and dinitrobenzene. In contrast to the reported formation of nitrite in the decomposition of nitrogenous compounds by the oxygen-flask combustion technique,[9] no nitrite was found in any of the tests described above.

METHOD

APPARATUS—

Nickel "fluorine bomb," capacity 2 ml—Obtainable from Charles W. Cook and Sons Limited, 97 Walsall Road, Perry Barr, Birmingham.

Conical flask with ground-glass stopper, capacity 150 *or* 200 *ml*—The type described by Haslam and Moses[10] is recommended.

Magnetic stirrer.

Microburette, capacity 10 *ml*—Conforming to B.S. 1428.[11]

REAGENTS—

Sodium peroxide—M.A.R. grade.

Ethanol, absolute.

Barium perchlorate, 0·01 N, *aqueous*—Adjust to about pH 3·0 by adding perchloric acid.

Thorin indicator solution—Dissolve 25 mg of thorin in 5 ml of distilled water.

Methylene blue indicator solution—Dissolve 15 mg of methylene blue in 50 ml of water.

Cation-exchange resin, Amberlite IR-120(*H*)—Suitable pre-treatment of the resin has been described elsewhere,[5] but is given here for the convenience of the user. By the column method, wash about 400 g of the analytical-grade resin with about 700 ml of 3·0 N hydrochloric acid and then with 4 or 5 litres of water. Transfer the resin to a 1-litre flask, remove most of the water by decantation, and shake the resin thoroughly for several minutes with three successive 100-ml portions of ethanol. Remove the residual ethanol by repeated washing with water, and shake the resin vigorously with 400 ml of 0·5 N sodium hydroxide for about 10 minutes. Decant the slightly turbid solution, wash the resin with water until all suspended fine particles have been removed, and filter on a Buchner funnel. Press the resin between filter-papers, and store it in a glass stoppered bottle sealed with adhesive tape.

PROCEDURE—

Fusion of the sample with sodium peroxide—Place in the dry bomb 0·5 g of powdered sodium peroxide, a suitable weighed amount of the sample (5 to 15 mg) and a further 0·5 g of sodium peroxide. Close the bomb, and mix the contents by rotation. Heat the bomb in a muffle furnace for 3 minutes at 650° C. Cool the bomb, remove the lid, and extract the fusion product by placing the bomb in a small beaker containing 10 to 15 ml of water and warming until effervescence ceases. Remove the bomb, rinse it with water and then rinse the lid. Transfer the combined solutions quantitatively to a 50-ml calibrated flask, and dilute to the mark.

Removal of sodium from the fusion-product extract, and titration of the sulphate ion—Place about 30 g of the cation-exchange resin in a conical flask. Add about 25 ml of ethanol, shake the stoppered flask for about 1 minute, remove the ethanol by decantation, and repeat the operation with a further 20 ml of ethanol. Transfer 25 ml of the fusion-product extract to the resin, shake for about 5 minutes, and decant the solution into a suitable conical titration flask containing a magnetic stirrer bar. Rinse the resin with four successive 25-ml portions of ethanol; add all washings to the contents of the titration flask. Add 0·1 ml each of thorin and methylene blue indicator solutions, and titrate with 0·01 N barium perchlorate to a pink end-point colour persisting for about 20 seconds. Carry out a blank determination in the same manner, omitting only the sample. The blank value should lie between 0·1 and 0·2 ml.

PRECAUTIONS—

1. When the sodium peroxide fusion is carried out as described, only a small amount of insoluble matter should be found in the aqueous extract of the fusion product, but this material can interfere with the detection of the end-point of the titration. For the best results the extract should be set aside overnight, so that a clear portion can be withdrawn for titration.

2. End-points in the barium perchlorate titration may be unsatisfactory if the ethanol-washed resin is set aside for more than about 2 hours before use. If such a delay is unavoidable, the resin should be washed once more with about 20 ml of ethanol immediately before use.

3. With some batches of resin it has been observed that, after pre-treatment in the prescribed manner, unsatisfactory end-points are obtained in the titration of the resin-treated sample solution. If this defect is found with any portion of a given batch of resin, it is recommended that all subsequent portions should be vigorously shaken with about 25 ml of 0·5 N sodium hydroxide for about 10 minutes, and then washed with water and ethanol in succession before use.

I thank Mr. C. G. Scott for assistance with the experimental work.

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Automatic Thermometric Titration*

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The usefulness and applications of thermometric methods of chemical analysis are discussed, with particular reference to automated routine volumetric analysis. Titrations have been performed with a constant-delivery-rate syringe burette, and the temperature change of the titrand has been followed by means of a thermistor bridge feeding a recording millivoltmeter. The method is widely applicable and can be made sufficiently rapid to make strict ambient temperature control unnecessary. Consecutive end-points of different components of mixtures may be detected in one titration by using single- or two-component titrants.

It is well known that, when a chemical reaction occurs, energy changes take place resulting in the evolution of heat into, or the absorption of heat from, the surrounding medium. Thus, for example, when an acid is neutralised by adding alkali, heat is evolved equivalent to the heat of formation of water, and the temperature of the solution rises.

Similarly, during the chelation of aluminium or magnesium ions with tetrasodium ethylenediaminetetra-acetate heat is absorbed and the temperature of the mixture falls. The energy changes occurring in such reactions are, of course, proportional to the amount of substance formed, and so might be used as a measure of this amount. Until recently little use appears to have been made of this parameter for analytical work,[1] the main reason, no doubt, being the availability of other more practical methods for determining the end-points of reactions, for example, by the use of coloured indicators or by changes in electrode potentials. The advent of the thermistor, a semi-conductor device having a high negative temperature coefficient of resistance, has, within the last decade, made it possible to measure rapidly, accurately and conveniently small changes in temperature, and thus to record the course of rapid chemical reactions. Several workers in America[2,3,4,5,6] have suggested that this principle of measuring heat changes or "thermometric titration" could be of great value, since the end-point of a reaction followed in this way would not be obscured by other effects, such as colour of solution or poisoning of electrodes, etc. If suitable precautions were taken, side effects due to dilution or stirring could be eliminated, since these would be included in the standardisation process.

An apparatus based on a circuit published by Jordan and Alleman[4] was constructed in this laboratory, and the results obtained with it were encouraging. For example, it was found possible to follow on a recording millivoltmeter the temperature changes occurring in many different types of reaction, including acid - alkali, oxidation - reduction, chelatometric and precipitation titrations.

INSTRUMENTATION

The earlier workers in the field of thermometric titration (or enthalpy titration as it is sometimes called) used a syringe burette driven by a synchronous motor which delivered titrant at a constant rate up to about 0·6 ml per minute. The reaction vessel was thermally insulated by being contained in a Dewar flask, and stirring was by means of a 600 r.p.m. glass stirrer. The molar concentration of the titrant was usually about 100 times that of the titrand to minimise volume changes and the effect of slight differences in temperature of the two solutions. (Under these conditions ±0·3° C difference could be tolerated.) Titration times of about 2 minutes were used, and the temperature changes were followed on a recording millivoltmeter fed from a bridge circuit incorporating a thermistor. The curve obtained showed a temperature ordinate and a titre abscissa. Tangents to the curve were drawn to simplify measurement of the length of the chart between the initial and equivalence-points. This method eliminated recorder-response time lags and other variables, since they were self-compensating.

Zenchelsky and Segatto in their paper on derivative thermometric titrations[3] pointed out that an increase in the rate of addition of titrant would be advantageous, since this would increase the rate of rise of temperature and thus permit the first or second differential of the temperature - time curve to be used to obtain sharper end-point determination. They

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* Communication No. 2349H from the Kodak Research Laboratory.

suggested, however, that a limit to such an increase in rate of titrant addition would be set by problems of heat transfer and gave 2 ml per minute as their estimate of the limit as applied to a volume of about 25 ml of titrand solution. During some earlier work in this laboratory, when high-speed mixing was required, it had been found that a vibrator, driving a Perspex paddle drilled obliquely with several holes, was much more effective and convenient than other methods tried. Accordingly, this method (see Fig. 1) was adopted in our work on thermometric titration, the thermistor being made the support for the paddle and thus also vibrating in the titrand solution during the reaction. Under these conditions, it has in fact been found that the efficiency of stirring allows a titrant addition rate of up to 10 ml per minute. This means that a complete titration may be carried out in less than 20 seconds, and, in consequence, heat losses or gains due to ambient temperature changes or small differences between temperatures of titrant and titrand are insufficient to interfere with end-point detection. The use of a Dewar flask to protect the reaction vessel is no longer necessary, and construction of the apparatus is simplified. In the first experiments a Metrohm syringe burette giving a titrant addition rate of 10 ml per minute was used. This was unsuitable for modification to meet our requirements in other respects, and it was later decided to construct an automatic syringe burette specifically designed for the type of titration envisaged.[7] This accommodated a standard 5-ml glass syringe giving a delivery rate of 7·0 ml per minute. The temperature changes and titration times (which were proportional to volume of titrant added) were followed on a recording millivoltmeter in a similar manner to that used by earlier workers, though at a later stage in our work it was found more convenient to replace the normal bridge circuit by one giving constant sensitivity over a temperature range of 15 to 30° C.[8] The chart speed used by Jordan and Alleman was 1 inch per minute, and a titration was usually of about 2 minutes duration, so that the accuracy depended on careful measurement of about 2 inches of record. In our work, the titrations being of only 10 to 20 seconds duration, a much greater chart speed was necessary, and speeds of up to 30 inches per minute were used. Thus up to 10 inches of record were obtained per titration, and this allowed an accuracy of about ±0·5 per cent. in measurement of titre. For convenience of illustration in this paper, chart speeds of 6 inches per minute were used giving results similar to those of Jordan and Alleman[4] (on the curves, 1 ml of titrant is equivalent to approximately 8·6 seconds). The time involved in obtaining quantitative results from the recorder charts is, however, out of proportion to that taken by the titration itself and, although this is relatively unimportant in investigative work, it would greatly detract from the value of the method for routine operation. For this reason it was considered necessary to develop apparatus that would permit results to be obtained in digital form. Details of the construction of a prototype automatic digital thermometric titrator, which is simple to operate and maintain, have already been given.[7]

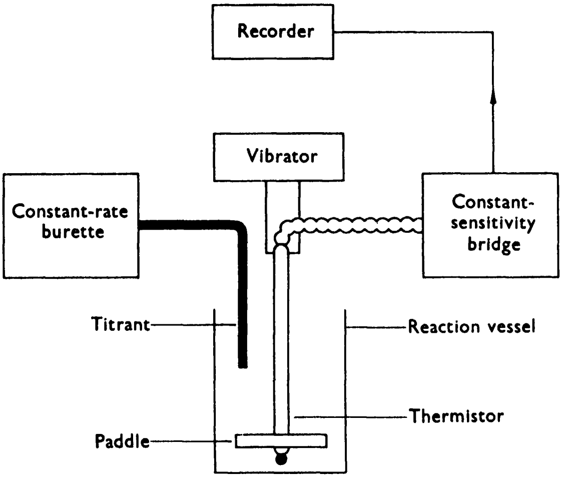

Fig. 1. Titration apparatus

ANALYTICAL APPLICATIONS

Turning now to various aspects of the analytical applications for which thermometric titration might be suitable, we may first make a general classification according to the rate of chemical reaction involved. Thus, in order to carry out direct titrations by this method, the rate of addition of titrant must be slow enough to allow it to be used up as it is added; otherwise the concentration would build up during a titration, and an incorrect end-point would be obtained. Fortunately, most inorganic reactions are ionic in character and, at the dilutions in which we are interested, take place almost instantaneously, subject to the limitations imposed by mixing. However, should a slow reaction be involved it will usually be possible to determine the concentration of a reactant by adding an excess of the titrant, allowing time for the reaction to be completed (if necessary accelerating this by heating) and then back-titrating with a suitable rapidly-reacting reagent. Back titration may also be of value when the excess of a reactant giving low-energy change may be determined by titration with another reactant giving a much greater energy change. Subject to this restriction then, the only other limitation to the use of the method for any reaction appears to be the amount of the energy change involved. For the prototype digital thermo-titrator this limitation was found to correspond to a rate of temperature change of 0·01° C per second. By using a sensitive recording millivoltmeter, qualitative results have been obtained for energy changes of about the same order. If suitable concentrations of reactant are selected, all the well-known types of inorganic reaction are included in this range, although there may be some individual ones for which our instrument is not sufficiently sensitive. It must be stated that the medium in which reactions are carried out is unimportant provided it does not attack the glass protective coating of the thermistor. Thus a wide field of non-aqueous reactions[1] is opened for study, and this procedure may prove invaluable for organic chemical assay. Some typical reactions, which are illustrated in Figs. 2 to 7, will now be considered. For convenience of discussion these may be classified into groups.

1. NEUTRALISATION OF ACID BY ALKALI—

The neutralisation of a strong acid by a strong alkali is typified by curve B of Fig. 2. For a dibasic alkali, curve C, there are two equivalence-points, the first (a) corresponding to neutralisation of one equivalent of acid, *i.e.*, the formation of the acid salt, in this instance the hydrogen carbonate, and the second (*b*) to complete neutralisation, *i.e.*, the formation of the neutral salt. For a strong acid and weak alkali titration, the equivalence-point is less distinct, as shown in curve D.

2. PRECIPITATION (DOUBLE DECOMPOSITION)—

Curve A of Fig. 2 illustrates the exothermic reaction between hydrochloric acid and silver nitrate. It will be seen that the heat produced in this reaction is greater than that due to neutralisation of an equivalent amount of hydrochloric acid. Further examples are shown in Fig. 7, and these will be dealt with under "Mixed Titrants."

3. OXIDATION - REDUCTION—

Curves A, B and C of Fig. 3 are typical of oxidation - reduction titrations and show the reaction of 0·05 M potassium permanganate with sodium sulphite, acidified potassium iodide and acidified ammonium ferrous sulphate solutions, respectively. This type of reaction gives the greatest observed heat change, which for sodium sulphite is about 6 times that for an equivalent neutralisation reaction.

4. CHELATION—

Probably the most widely exploited chemical in inorganic analysis in recent times is the tetrasodium salt of ethylenediaminetetra-acetic acid, Na_4Y, and this has been chosen to illustrate some of the interesting possibilities of the thermometric method in this field. Thus, in Fig. 4, curve B illustrates the reaction of Na_4Y with barium nitrate. This is a straightforward exothermic reaction giving a well-defined end-point corresponding to the formation of $Na_2[BaY]$. Curve A, for the reaction with silver nitrate, shows three equivalence-points (c), (d) and (e), corresponding to $Na[Ag_3Y]$, $Na_2[Ag_2Y]$ and $Na_3[AgY]$. This reaction is also an exothermic one, but, as has been pointed out by earlier workers, many cations give an endothermic reaction with Na_4Y, *e.g.*, magnesium and aluminium. It has also been shown that, on adding Na_4Y to a mixture of such cations as calcium and magnesium, the whole of the calcium is chelated before the magnesium starts to react, giving a thermometric titration curve showing a rise in temperature due to the first reaction and then a fall due to the second. We have found experimentally that, as perhaps might be expected, the sequence of reaction with Na_4Y in any mixture of cations corresponds to the sequence of the stability constants of the chelates concerned. The sequence we have found is listed in Table I, the most reactive metal ion (mercuric) being placed at the top and the least reactive (potassium) at the bottom. It will be noticed, however, that there is no obvious relation between sequence of reaction and direction of energy change. Whereas the majority of the reactions are exothermic, others are endothermic and these can, therefore, be used to separate the titration of metals on either side of them in the list. For example, mixtures of ferric iron, aluminium, calcium, magnesium and barium have been separated in a single titration as shown in Fig. 4, curve D. The equivalence-points (h), (j), (k), (l) and (m), do not, however, correspond exactly to the expected values, since, under the experimental conditions used, interactions occur. These interactions may be due to different rates of reaction or ionisation, which would lead to substitution in the manner described under "Substitution Reactions."

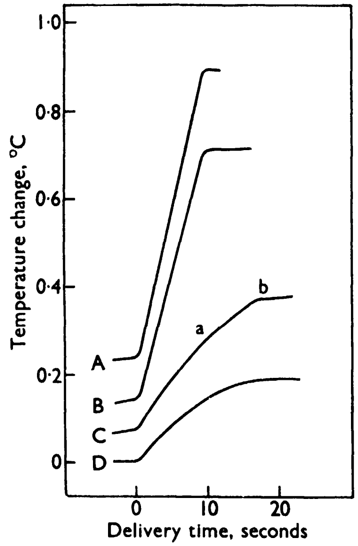

Fig. 2. Titrations with M hydrochloric acid of 20 ml of titrand containing 1 mmole of reactant: curve A, silver nitrate; curve B, sodium hydroxide; curve C, sodium carbonate; curve D, sodium tetraborate

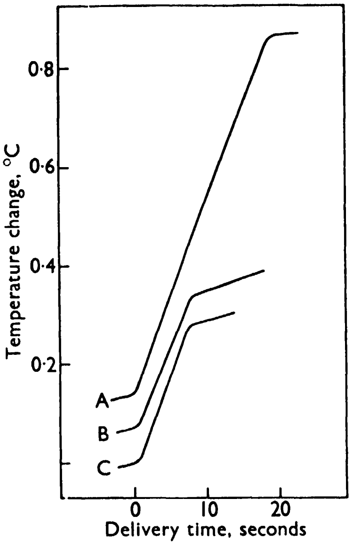

Fig. 3. Titrations with 0·05 M potassium permanganate of 20 ml of titrand containing 0·2 mmole of reactant: curve A, sodium sulphite; curve B, potassium iodide + sulphuric acid; curve C, ammonium ferrous sulphate + sulphuric acid

Some Na_4Y reactions do not give a sharp turn-over at the end-point, and it is often possible (by adding a second constituent to the titrand solution, whose heat of reaction is of opposite sign and which reacts after the solution being determined) to increase the rate of heat change at the end-point and so to achieve sharper detection. Curve C of Fig. 4 demonstrates the improved barium end-point (f) (compare curve B) obtained by adding ammonium nitrate solution, which itself gives an extremely poor end-point (g).

5. SUBSTITUTION REACTIONS—

It can be seen from the experimental results shown in Table I, indicating the relation between sequence of reaction and stability constants for EDTA chelates, that the metal ion in a solution of any chelate may be displaced by any other metal ion higher in the Table. It will further be seen that there are four possible combinations of substitution reactions of chelates which can occur. Thus, the metal ion in either an exothermally or endothermally formed chelate may be replaced by either an endothermally or exothermally reactive metal ion. Examples of these reactions are shown in Figs. 5 and 6.

The formation of calcium chelate is an exothermic reaction, so that when the chelate breaks down during substitution this heat appears in an endothermic form, while simultaneously the copper chelate is being formed in an exothermic reaction. Since curve B in Fig. 5 shows a rise in temperature, the conclusion is drawn that the heat of formation of the copper chelate is greater than that of the calcium chelate, although the types of reaction are similar.

On the other hand, replacement of calcium by aluminium, as illustrated in curve B of Fig. 6, gives an endothermic reaction with a greater heat change than that associated with the direct chelation of aluminium, although a poor end-point (o) is obtained.

TABLE I

SEQUENCE OF REACTION OF CATIONS WITH EDTA TETRASODIUM SALT AND PUBLISHED VALUES FOR STABILITY CONSTANTS (Log K_a)

Cation	Thermal change	Stability constants
Mercuric	Exothermic	21·8
Stannic	Exothermic	– -
Ferric	Exothermic	25·1
Cupric	Exothermic	18·3
Nickelic	Exothermic	18·6
Plumbous	Exothermic	18·0
Cadmium	Exothermic	16·5
Zinc	Exothermic	16·3
Cobaltous	Exothermic	16·1
Ceric	Exothermic	—
Aluminium	Endothermic	16·1
Ferrous	Exothermic	14·4
Uranyl	Endothermic	—
Manganous	Exothermic	14·0
Calcium	Exothermic	10·6
Beryllium	Endothermic	-
Magnesium	Endothermic	8·7
Chromic	Endothermic	—
Strontium	Exothermic	8·6
Barium	Exothermic	7·7
Silver..	Exothermic	7·3
Ammonium	Endothermic	—
Lithium	Endothermic	2·8
Potassium	Endothermic	—

The formation of magnesium chelate is an endothermic reaction, and replacement by copper results in a much greater heat change, as shown in curve A of Fig. 5, than that associated with the direct chelation of copper. In this reaction a good end-point is obtained. On the other hand, replacement of magnesium by aluminium, as shown in curve A of Fig. 6, gives a smaller heat change than for the direct chelation of aluminium. It may be concluded that the heat of formation of the aluminium chelate is greater than that of magnesium chelate. Once again a poor end-point (n) is obtained.

It is obvious that this method could be extended to facilitate analyses in which the heat of formation of the chelate is of low order, since, by suitable choice of the metal cation being displaced, much greater temperature changes can be observed.

6. MIXED TITRANTS—

Under "Chelation," the simultaneous determination of several components of a mixture of metal cations by titration with a single titrant was discussed. Similar results can be obtained for anions; for example, a mixture of iodide, bromide and chloride titrated with silver nitrate shows equivalence-points in that order, as would be expected. In some reactions, it is possible to make use of an extension to such a scheme of analysis by using a mixed titrant. An example of this is shown in Fig. 7, where a mixture of silver and thorium nitrates has been used to titrate all four halides, both separately and in a mixture. It will be seen from curve A that, at the start of the titration, fluoride and iodide react simultaneously to produce a rate of temperature rise equivalent to the sum of the separate reactions. When all the fluoride has been used up, equivalence-point (p), the iodide continues to react until it is used up at (q), followed by the bromide (r) and chloride (s).

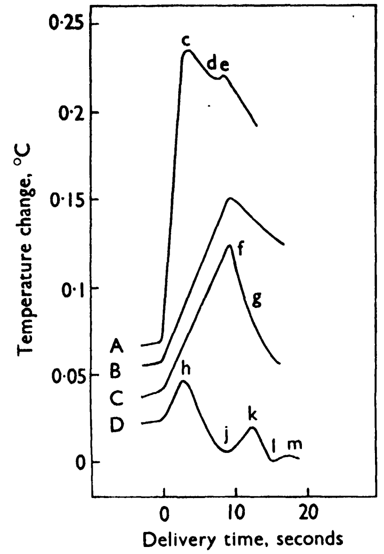

Fig. 4. Titrations with M tetrasodium EDTA of 20 ml of titrand containing: curve A, 1·0 mmole of silver; curve B, 1·0 mmole of barium; curve C, 1·0 mmole of barium + 1·0 mmole of ammonium; curve D, 0·3 mmole of iron$^{\text{III}}$ + 0·3 mmole of aluminium + 0·8 mmole of calcium + 0·25 mmole of magnesium + 0·15 mmole of barium

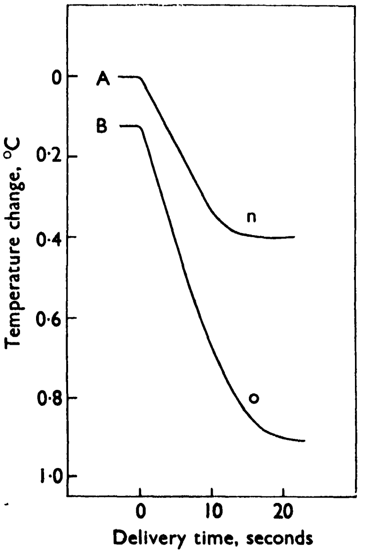

Fig. 6. Titrations with M aluminium nitrate of 20 ml of titrand containing 1 mmole of reactant: curve A, disodium magnesium - EDTA complex; curve B, disodium calcium - EDTA complex

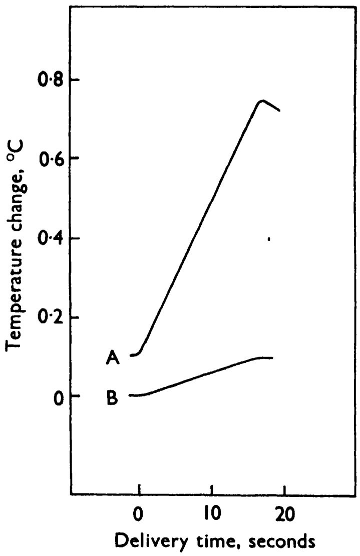

Fig. 5. Titrations with M cupric nitrate of 20 ml of titrand containing 2 mmole of reactant: curve A, disodium magnesium - EDTA complex; curve B, disodium calcium - EDTA complex

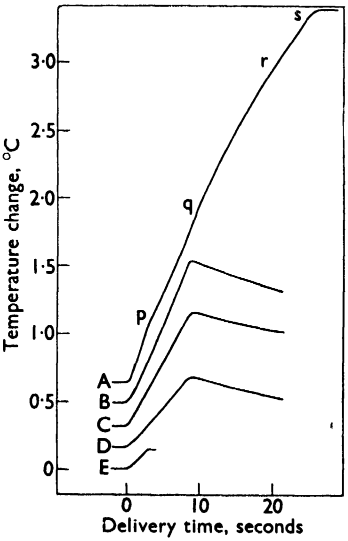

Fig. 7. Titrations with M thorium nitrate + silver nitrate of 20 ml of titrand containing 1 mmole of reactant: curve A, sodium fluoride + sodium chloride + sodium bromide + sodium iodide + nitric acid; curve B, sodium iodide; curve C, sodium bromide; curve D, sodium chloride; curve E, sodium fluoride + nitric acid

QUANTITATIVE DATA—

Although quantitative results can usually be more simply and rapidly obtained by using the automatic digital titrator, it is obviously desirable to make some reference to the method involving the use of a recorder to plot the thermal changes occurring during a titration. The conditions of experiment with respect to rate of addition of titrant, stirring and temperature sensing were the same as for the digital method. It follows that the quantitative data obtained by the latter method may also be taken as representative of the accuracy and reproducibility of the recorder method, provided that the actual recorder used has suitable sensitivity, rapid response and a sufficiently fast chart speed to permit accurate measurements to be taken on the curves obtained. It should be noted that the sharpness or otherwise of the curve of the record at the end-point of a reaction does not unduly influence the precision with which it may be located, provided that the straight line portions are long enough to permit tangents to be drawn accurately. This is a limitation for mixtures containing only a small proportion of one component, but the fact that such a minor component would usually be detected might nevertheless be an advantage and allow the analyst to use an alternative method of determination.

The results obtained when a chart speed of 8 inches per minute and a titrant delivery rate of 6·92 ml per minute were used are shown in Table II.

TABLE II

PRECISION OF TITRATION

Titrand	Titrant	Replicates	Mean titre, mm	Standard deviation, %
20 ml of 0·1 M $Cu(NO_3)_2$	M EDTA (Na_4)	11	60·28	±0·3
20 ml of 0·1 M NaOH	M HCl	11	57·06	±0·4
10 ml of 0·2 M $AgNO_3$	M HCl	11	58·39	±0·6
20 ml of 0·1 M I_2(in KI)	M $Na_2S_2O_3$	12	62·22	±1·3

CONCLUSIONS

It is apparent from the foregoing that the method is widely applicable. Thus, whereas most of the well-known volumetric methods can be carried out thermometrically, most of the determinations that are normally performed gravimetrically are also possible. In some instances several components can be determined by a single titration, thus avoiding the separation process that might otherwise be necessary. Alternatively, an extra component may sometimes be added to a mixture to provide end-point discrimination between two other components, again avoiding the necessity of making a physical separation. The saving in the time taken for analysis by precipitation reactions as compared with the gravimetric method will be obvious, but it is also considerable when compared with straightforward volumetric analysis with the use of indicators.

In comparison with potentiometric titrations, the thermometric method is simpler in that one thermistor takes the place of a series of different types of electrode. It must, however, be emphasised that, at this stage of instrumentation, it is not possible to work with such low concentrations as are possible with the former method.

It has been shown that the sequence of reaction of EDTA with a mixture of cations corresponds to the sequence of the stability constants of the chelates concerned. Similarly, for precipitation reactions in which there is a common ion, the sequence corresponds to that of the solubility products of the precipitates. It is therefore possible, in most instances, to predict from available data whether or not a thermometric titration method will be suitable for any given analytical problem. In this way, reagents that are not usually even considered for titrimetric work may be found to be suitable for particular applications. For example, magnesium chloride might be used for determining carbonate in a mixture of carbonate and hydrogen carbonate since magnesium hydrogen carbonate is sufficiently soluble not to interfere with the carbonate end-point. This titrant could also be used to back-titrate most cations, after adding the tetrasodium salt of EDTA, since the magnesium - EDTA complex has a fairly low stability constant and would not displace cations with higher stability constants.

Finally, as a result of the work described here and elsewhere, it is our belief that thermometric titration will play an important part in the future development of analytical methods, not only in the inorganic but also in the organic field.

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Determination of Specific Surface Area by Air-permeation at Low Porosities

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The average particle diameter of a powder can be determined with the Fisher sub-sieve sizer, as normally operated, at porosities within the range 0·80 to 0·40. The resulting figure for this diameter varies with the porosity of the powder bed, inadequate compression of the sample yielding a high result. A similar variation occurs with other air-permeation methods. Certain samples probably require compression to porosities below 0·40 to achieve uniform porosity and a consistent result. A simple method for extending the porosity range of the Fisher instrument below 0·40 is described.

MANY authors[1,2,3,4] have observed that the average particle diameter of powders, determined by air-permeation, varies with the porosity of the sample bed, lower values for this diameter being obtained at low porosities. We have confirmed, for several samples of griseofulvin and other antibiotics, that this phenomenon also occurs with the air-permeation apparatus described by Gooden and Smith[5] and available commercially as the Fisher sub-sieve sizer (Fisher Scientific Company, Pittsburg, Pa., U.S.A.; Kek Ltd., Ancoats, Manchester 12).

It is therefore evident that air-permeation does not yield a unique value for the average particle diameter of a powder. To overcome this difficulty, we prefer to test the sample at various porosities and to record as the diameter of the sample the minimum value found. For this purpose the Fisher sub-sieve sizer is more convenient than other types of apparatus, because it incorporates a device for compressing the sample to successively lower porosities and a chart from which the porosity and average particle diameter can be read directly.

Some samples, however, can readily be compressed to porosities lower than the minimum, 0·40, provided for on the Fisher chart. The main purpose of this paper is, therefore, to describe means whereby such samples can be tested at lower porosities with the existing chart.

METHOD

The instrument is assumed to obey the Gooden and Smith[5] equation, which can be expressed as—

$$d_{\text{vs}} = \frac{cLX}{(AL - X)^{3/2}} \cdot \sqrt{\frac{F}{P - F}} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where d_{vs} is the average particle diameter (volume-surface mean diameter) in microns, c is the instrument constant (3·80), L is the height of the compressed bed of powder (cm), X is the volume of the sample excluding bed porosity (c.c.), A is the cross-sectional area of the sample tube (1·267 sq. cm), F is the pressure difference across the flowmeter resistance (g per sq. cm), and P is the instrument air pressure (50 g per sq. cm).

The instrument chart, however, is designed to be used with a standard sample volume, X, of 1 c.c. It is, therefore, calibrated according to the equation—

$$d_{\text{vs}}' = \frac{cL}{(AL - 1)^{3/2}} \cdot \sqrt{\frac{F}{P - F}} \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

where d_{vs}' is the diameter indicated on the chart and corresponds to the average particle diameter only when the instrument is used as intended, *i.e.*, when $X = 1$.

The chart also indicates the bed porosity, ϵ', in accordance with the equation—

$$\epsilon' = 1 - 1/AL \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

This equation also is correct only when $X = 1$ since, in general, the bed porosity, ϵ, is given by—

$$\epsilon = 1 - X/AL \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

If a different sample volume is used, so that X is not unity, the average particle diameter can nevertheless be calculated from the diameter indicated on the chart. Comparison of equations (1) and (2) shows that the average particle diameter, d_{vs}, will be given by—

$$d_{\mathrm{vs}} = X\left(\frac{1 - 1/AL}{1 - X/AL}\right)^{3/2} d_{\mathrm{vs}}' \quad \ldots \quad (5)$$

and hence, after substitution of equations (3) and (4) in equation (5), by—

$$d_{\mathrm{vs}} = X(\epsilon'/\epsilon)^{3/2} d_{\mathrm{vs}}' \quad \ldots \quad (6)$$

Similarly, the bed porosity, ϵ, can be calculated from the indicated porosity, ϵ', by means of the equation—

$$\epsilon = 1 - X(1 - \epsilon') \quad \ldots \quad (7)$$

We have found that the value of X must lie between about 1·2 and 1·3 to extend the range of ϵ down to the minimum porosities attainable with some samples we have used. If ϵ' is set at intervals of 0·04 and X is 1·25 c.c., ϵ will form a series at intervals of 0·05, as shown in Table I. This has the advantage that no interpolation is necessary when setting ϵ' on the instrument chart or plotting ϵ on a graph of the results.

The weight of sample required to give a volume of X c.c. is $X\rho$ g, where ρ is the density of the particles. For smooth particles containing no internal pores, ρ is the density of the solid material comprising the particles. Since, in general, the air flows around each particle but not through internal pores, the appropriate density of a porous particle is equal to its weight divided by its volume including internal pores.

The powders with which we have worked consisted of simple crystals, or fragments of crystals, with no internal faults or pores visible under a microscope. We have assumed, therefore, that the appropriate particle density is that measured by displacement of liquid in a specific-gravity bottle.

PROCEDURE—

Take a weight (in grams) of sample equal to the density × 1·25. Compress the weighed sample to the sample height curve of the Fisher chart at a high value of indicated porosity from Table I. Read the indicated diameter on the chart, and multiply the result by the corresponding diameter factor from the Table. Compress the sample to successively lower porosities until the corrected diameter reaches a minimum.

TABLE I

DIAMETER CORRECTION FACTORS AND THE CORRESPONDING VALUES OF INDICATED AND CORRECTED POROSITY

The sample volume, X, = 1·25 c.c.

Indicated porosity, ϵ'	Corrected porosity, ϵ	Diameter factor, $X(\epsilon'/\epsilon)^{3/2}$
0·80	0·75	1·377
0·76	0·70	1·414
0·72	0·65	1·457
0·68	0·60	1·508
0·64	0·55	1·569
0·60	0·50	1·643
0·56	0·45	1·735
0·52	0·40	1·853
0·48	0·35	2·008
0·44	0·30	2·220
0·40	0·25	2·530

EXPERIMENTAL

The Fisher instrument was calibrated to conform with equation (1) by checking the accuracy of the constants c, A and P and the variables L and F. Details of the calibration method, which involves the use of a capillary tube of accurately known dimensions, are contained in a paper being prepared for publication.

The validity of the correction method (equation (6)) was demonstrated by testing a sample of griseofulvin at the normal sample weight level and at the × 1·25 level.

The normal weight sample was compressed to successively lower porosities over the range 0·65 to 0·40, and corresponding readings of average diameter were obtained. The over-weight sample was similarly tested over the range 0·68 to 0·41 indicated porosity, corresponding to 0·60 to 0·26 bed porosity. Light compression in a screw clamp was necessary to attain porosities below 0·35; because this might have crushed some of the particles and altered the average diameter, the compressed plug was removed from the tube at the end of the run, broken up through a 40-mesh sieve and re-tested on the instrument at the × 1·25 level.

RESULTS

The values for average particle diameter for the three determinations were plotted against porosity (see Fig. 1). The various points obtained from the two levels of sample weight lie close to a single curve and thus confirm the validity of the method. The points resulting from the repeat determination on the previously compressed plug also fall close to the original curve at the low porosities. This demonstrates that the slope of the curve is not due to the particles being crushed by compression.

The shape of curve shown in Fig. 1 is typical of our results in testing many samples of griseofulvin and other antibiotics. The average diameter falls more or less rapidly at first and then levels out to a minimum when tight compression has been achieved. Doubtless, this effect is due, at least in part, to the tendency of fine powders to form lumpy aggregates, as discussed below. Rigden[6] has stated that determinations of air-permeation on such powders will be in error at high porosities if the bed of powder contains aggregates of lower porosity than the matrix in which they are embedded. Until the bed is compressed to a uniform porosity equal to that of the aggregates, air will pass more readily around than through the aggregates. Thus the instrument reading will reflect the diameter of the aggregates rather than that of the individual particles.

Carman and Malherbe[4] found a similar tendency for the diameter of fine powders to become constant at low porosities. For each sample there was a different "normal" porosity range within which the powder was compressed uniformly enough to give a constant result. They found a normal porosity range of about 0·5 to 0·4 for samples of uniform particle size, but concluded that for non-uniform samples, in which small particles fit into the voids between large ones, normal porosity could well be 0·3 or less.

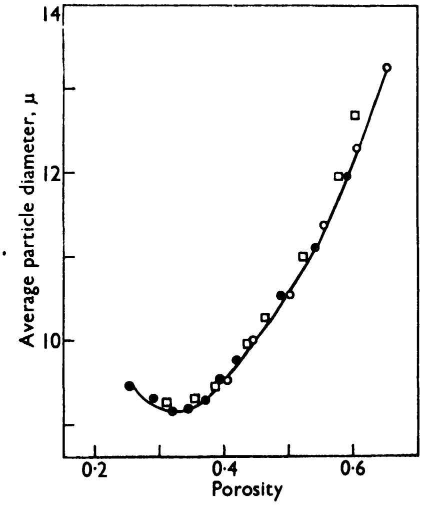

Fig. 1. Average particle diameter of a sample of griseofulvin as determined in the Fisher sub-sieve sampler: ○, on normal weight of sample; ●, on over-weight sample, results corrected as shown in Table I; □, compressed plug, from previous determination at porosity 0·26, broken up through sieve and re-tested

This accords well with our experience of griseofulvin samples. We have found normal porosities as high as 0·53 for samples of uniform particle size and as low as 0·25 for non-uniform material.

With further reduction of porosity, our curves usually show a slight rise in diameter after the minimum has been reached. This feature is absent from the results of Carman and Malherbe; because their samples were finer than ours, it is possible that they had greater difficulty in achieving a sufficiently tight compression.

This means that normal porosity is not to be regarded as a range within which the diameter is a constant, but as a point at which the diameter is a minimum. In this connection, as Carman and Malherbe have observed, there is a scatter of experimental points about a mean value of diameter at each porosity, so that results over a limited porosity range may give the impression that diameter is independent of porosity. In our work, experimental scatter was reduced by attention to the details of calibration mentioned above.

The question of which point on such curves corresponds to the true diameter raises complex issues, not only about uniformity of porosity but also about validity of the porosity function in the equation and the theory of air flow.[3] Discussion of these problems is beyond the scope of this paper, but further work on them is described in detail in a paper being prepared.

Meanwhile, for purposes of analytical control, there is a need to define a technique that will give a unique value for the average particle diameter of a powder. The American Society for Testing Materials' method for cement[7] meets this need by standardising the porosity of the compressed sample at 0·500. This is satisfactory, because cement is free-flowing and non-cohesive; the range of porosities that can be achieved with cement is limited.

With more cohesive powders, such as griseofulvin, it is possible to achieve a wider range of porosities, and the variation of average particle diameter with porosity is greater. Defining the average diameter as the minimum on the diameter - porosity curve not only provides the unique value required for analytical control, but avoids possible error due to channelling of air through an inadequately compressed bed when the sample has an unusually low normal porosity.

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The Quantitative Determination of Nitroglycerin by the Schultze - Tiemann Method

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The Schultze - Tiemann method for determining nitrogen content has been used for quantitatively determining nitroglycerin in powders and dynamites. The method is simple, rapid and gives accurate results; the presence of diphenylamine, diethyldiphenylurea, dimethyldiphenylurea, dipentylphthalate, dinitrotoluene or paraffin oil does not interfere.

In the analysis of explosives it is frequently necessary to determine the nitroglycerin content of powders and dynamites. Different procedures for the quantitative determination of nitroglycerin have been proposed; some depend on the preferential solvent action of carbon disulphide on the true nitro-compounds and similarly that of aqueous acetic acid (about 75 per cent.) on glycerol trinitrate,[1,2,3] the two liquids being nearly immiscible. Methods are used in which nitroglycerin is destroyed so that other compounds, such as nitro-compounds present in the mixture, can be subsequently extracted and determined; the loss in weight can be calculated as the amount of nitroglycerin.[4] This has been effected by reduction with ferrous chloride in concentrated hydrochloric acid[5] and also with ammonium sulphide.[4]

It has long been known that the percentage of nitroglycerin in admixture with other compounds can be deduced from the nitrogen content. This method will be applicable only if no substance is present that will evolve nitrogen in the determination, or if the amount of nitrogen due to other nitric esters or nitrates is known.[3] Several methods for determining the nitrogen content have been suggested.[6,7,8,9,10] The methods proposed by Silberaad, Phillips and Merriman[11] and Muraour[10] rely on the reduction of the nitrate groups of nitroglycerin by suitable reagents and then the determination of the nitrogen as ammonia. Excellent results were obtained by Muraour's method, but, because of the time required for a determination, it was not suitable for control analysis.[6]

The ferrous - titanous titration procedure proposed by Knecht and Hibbert[12] for analysing potassium nitrate was applied by Becker to glycerin trinitrate.[6] Becker's volumetric method for the quantitative determination of nitroglycerin consists in dissolving the extracted ingredients from the powder sample in acetic acid, adding an excess of ferrous chloride and hydrochloric acid, boiling the solution, and then titrating the resulting ferric iron with standard titanous chloride solution, ammonium thiocyanate being used as indicator. An atmosphere of carbon dioxide is maintained in the titration flask during the entire period. Although a somewhat complicated piece of apparatus is required, this method is the one most widely used in control and research laboratories[8]; small amounts of diethyldiphenylurea, diphenylamine or both do not interfere in the determination.

Other methods for determining nitrogen content have been proposed; *e.g.*, the nitrometer method[3] and the Schultze - Tiemann method[9] were early suggested as methods for the quantitative determination of nitroglycerin. These methods were neglected when it was found that certain compounds, *e.g.*, diphenylamine, diethyldiphenylurea and mononitrotoluenes, present in admixture with nitroglycerin could interfere in the determination and cause low results.[3,6]

During this investigation, trials have been made to determine the nitroglycerin content of powders and dynamites by the Schultze - Tiemann method, but low results were consistently obtained. The causes of these low results were thoroughly investigated and the official Schultze - Tiemann method was slightly modified; satisfactory results have subsequently been obtained. The procedure was simplified so as to be suitable for the control determination of the nitroglycerin content of powders and dynamites.

METHOD

APPARATUS—

The apparatus (see Fig. 1) consists of a long-necked Kjeldahl flask, A, of capacity about 200 ml connected to a one-way valve, B, and a delivery tube, C. The Kjeldahl flask is tightly fitted with a rubber stopper carrying a funnel, D, with a stopcock, E. The nitric oxide is collected over approximately 33 per cent. sodium hydroxide solution in a graduated burette, F, with a zero mark beginning from the stopcock, G.

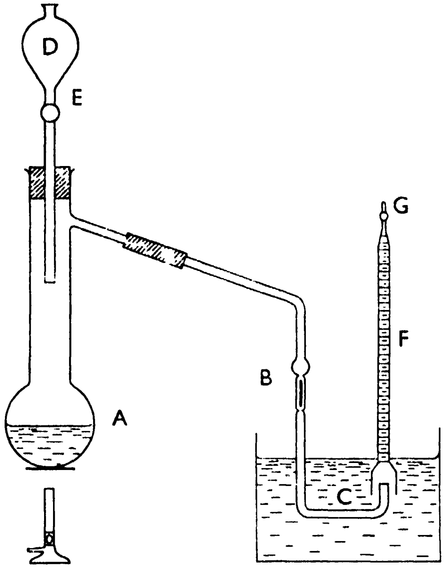

Fig. 1. Apparatus for determining the nitrogen content of explosives

REAGENTS—

Sodium hydroxide solution, approximately 33 *per cent.*

Ferrous chloride solution—Dissolve 120 g of powdered iron in 373 ml of hydrochloric acid, sp.gr. 1·19, and 227 ml of distilled water.

PROCEDURE—

Extract a weighed amount of the finely ground powder with diethyl ether in a Soxhlet apparatus for 24 hours.[4,8] Allow the ether in the extraction flask to evaporate, and dry the ether extract at 45° to 50° C for about 16 hours to constant weight. Cool, weigh, and calculate the ether extract as a percentage.

In a small weighing bottle weigh a sample of the ether extract (about 100 mg). Transfer a 20- to 30-mg sample (weighed by difference) to the flask. Introduce 30 ml of distilled water into the flask, and then add 10 ml of 0·1 N potassium permanganate. Tightly seal the flask by means of the rubber stopper carrying the funnel D. Open stopcock E and gently heat the flask with a bunsen burner. When the solution boils, close the stopcock E, and expel all air from the flask through valve B by continuing to boil the solution. To ensure that all the air is expelled, cool the apparatus, and introduce a small amount of water into the flask through stopcock E (care should be taken not to admit any air); then boil the solution again until no air collects over the sodium hydroxide in the burette.

After all the air has been expelled, introduce, successively, 20-ml portions of ferrous chloride solution and of diluted hydrochloric acid (1 + 1) into the flask from funnel D through stopcock E; take care not to admit any air into the reaction flask. Heat the flask, and collect the liberated nitric oxide over sodium hydroxide solution in the graduated burette F. After all nitric oxide has been expelled, repeat the process two or three times with 10-ml portions of ferrous chloride solution and of dilute hydrochloric acid and finally with distilled water until no further nitric oxide is liberated. Then transfer the burette to a cylinder filled with water, and, after 10 to 15 minutes, read off the volume, V, barometric pressure, P, and temperature, t; calculate the percentage by weight, G, of nitrogen in the sample from the expression—

$$G = \frac{V \times 100 \times 14{\cdot}01 \times (P - b) \times 273}{W \times 22389{\cdot}6 \times 760 \times (273 + t)}$$

where W = weight of sample taken and
b = water vapour pressure at t° C.

Hence calculate the nitroglycerin content of the sample from the expression—

$$\text{Nitroglycerin, \%} = \frac{\text{Percentage by weight of ether extract} \times G}{\text{Nitrogen content of nitroglycerin}}$$

NOTE—Blank experiments for determining the nitrogen content of nitroglycerin should be carried out in the same apparatus as used above. Before each set of determinations triplicate samples of commercial nitroglycerin should be analysed and the mean value taken as the nitrogen content; this value should accord with the theoretical (about 18·4 per cent.[13]). The Schultze - Tiemann apparatus should be checked periodically by testing with pure potassium nitrate as the sample.

RESULTS AND DISCUSSION OF THE METHOD

The Schultze - Tiemann method has been recommended by different workers for determining nitrate nitrogen.[7,15,16,17,18] It was once considered more suitable than the nitrometer method for analysing cellulose nitrate, particularly when the samples to be tested are in a physical condition such that they do not easily dissolve in sulphuric acid.[7,15] Results obtained by the nitrometer and Schultze - Tiemann methods were found to be in close agreement.[3,7,17,18]

During the investigation described here, formulated mixtures containing different amounts of nitroglycerol, dinitrotoluene, diethyldiphenylurea (Centralite I), dimethyldiphenylurea (Centralite II), dipentylphthalate (amylol) and paraffin oil were prepared as shown in Table I. The quantitative determination of nitroglycerin present in these formulated mixtures (see Table I), as well as certain types of powders and dynamites of known chemical compositions (see Table II), was carried out by means of the proposed method.

In the official Schultze - Tiemann method air is expelled from the decomposition flask by boiling water. After all the air has been expelled, ferrous chloride solution and dilute hydrochloric acid are added, and the reaction mixture is then boiled until all the nitric oxide has been liberated.

$$HNO_3 + 3FeCl_2 + 3HCl = 3FeCl_3 + 2H_2O + NO$$
$$KNO_3 + 3FeCl_2 + 4HCl = 3FeCl_3 + 2H_2O + KCl + NO$$

It is significant that when commercial nitroglycerin and the formulated mixtures (see Table I) were analysed by the procedure described in the official test, the results obtained were consistently lower than the theoretical. The nitrogen content of nitroglycerin was found to be about 16·5 per cent., whereas, when a sample was analysed by the nitrometer method, it was found to contain 18·35 per cent. of nitrogen. The percentages of nitroglycerin present in the formulated mixtures (see Table I) were also found to be 3 to 5 per cent. low; the nitrometer method also gave low results (by 3 to 4 per cent.).

On the other hand, when 10 ml of 0·1 N potassium permanganate were added to the distilled water present in the decomposition flask, before air is expelled from the flask, the results obtained were found to be satisfactory. Commercial nitroglycerin (when permanganate solution was added) was found to contain 18·4 to 18·45 per cent. nitrogen; a value that accords with the theoretical.[13] Further, it is apparent that the glycerin found by analysis in the formulated mixtures closely corresponds with the true value. When the method was applied to some types of powders and dynamites of known chemical compositions, the results were found to be satisfactory (see Table II).

The nitrogen contents of cellulose nitrate and of pure potassium nitrate were determined by means of the official Schultze - Tiemann method (without addition of permanganate) and the results (13·76 and 12·23 per cent. of nitrogen, respectively) were found to be in close agreement with the nitrometer values (13·81 and 12·26 per cent., respectively); this was not so when nitroglycerin was analysed. This may be related to the fact that glycerin trinitrate has an appreciable vapour pressure at 100° C[19,20] and part of the loss during the determination of nitrogen by the Schultze - Tiemann method (without addition of permanganate) may have been caused by steam distillation of the ester during the displacement of air from the decomposition flask. When potassium permanganate is added it oxidises glycerin trinitrate to nitric acid,[12]

$$C_3H_5(NO_3)_3 + 3\tfrac{1}{2}O_2 \longrightarrow 3HNO_3 + 3CO_2 + H_2O$$

and thereby minimises the vaporisation of nitroglycerin when the air is expelled from the flask.

TABLE I

DETERMINATION OF NITROGLYCERIN IN FORMULATED MIXTURES

Composition of mixture, %	Nitroglycerin found, %	Mean, %	Error, %
Nitroglycerin, 71·06; diphenylamine, 3·24; Centralite I*, 6·70; Amylol†, 19·0	71·08, 71·09, 71·05, 71·13	71·09	+0·03
Nitroglycerin, 59·02; dinitrotoluene, 30·00; diphenylamine, 5·40; Centralite I*, 5·58	59·02, 59·03, 59·07, 59·04	59·04	+0·02
Nitroglycerin, 51·78; diphenylamine, 20·00; Centralite II‡, 10·00; Amylol†, 18·22	51·84, 51·80, 51·77, 51·82	51·81	+0·03
Nitroglycerin, 79·8; diphenylamine, 7·0; Centralite I*, 4·5; Centralite II‡, 2·0; Amylol†, 6·7	79·86, 79·85, 79·76, 79·90	79·84	+0·04
Nitroglycerin, 32·97; diphenylamine, 4·00; Centralite II‡, 4·00; dinitrotoluene, 34·03; Amylol†, 15·00; paraffin oil, 10·00	33·05, 33·02, 33·01, 32·95	33·01	+0·04

* Diethyldiphenylurea.
† Dipentylphthalate.
‡ Dimethyldiphenylurea.

TABLE II

DETERMINATION OF NITROGLYCERIN IN POWDERS AND DYNAMITES

	Nitroglycerin found by—		
Specified composition of sample, %	Nitrometer method, %	Modification of Muraour's method,[4] %	Schultze - Tiemann method, %
Nitroglycerin powder I—			
Nitroglycerin, 27·1 ± 0·5; nitrocellulose, 64·0 ± 1·0; diphenylamine, 1·0 + 0·2; Centralite I*, 2·0 ± 0·2; Amylol†, 4·65 ± 0·2; petroleum jelly, 1·45 ± 0·2	24·3, 23·9, 23·5 (Mean 23·9)	27·09, 27·23, 27·40 (Mean 27·24)	27·30, 27·32, 27·32 (Mean 27·31)
Nitroglycerin powder II—			
Nitroglycerin, 26·0 ± 0·5; nitrocellulose, 68·8 ± 1·0; diphenylamine, 1·2 ± 0·2; Centralite I*, 0·5 ± 0·2; Amylol†, 3·5 ± 0·5	23·0, 23·3, 22·92 (Mean 23·07)	26·05, 26·10, 26·15 (Mean, 26·10)	26·10, 26·15, 26·18 (Mean, 26·14)
Nitroglycerin powder III—			
Nitroglycerin, 29·6 ± 0·5; nitrocellulose, 66·0 ± 1·0; diphenylamine, 1·0 ± 0·2; Centralite I*, 1·0 ± 0·2; Amylol†, 1·0 ± 0·2; petroleum jelly, 1·4 ± 0·2	26·6, 25·9 (Mean 26·25)	29·86, 29·80, 29·70 (Mean, 29·79)	29·72, 29·75, 29·80 (Mean, 29·76)
Extra dynamite I, ether extract—			
Nitroglycerin, 20 ± 2; trioil, 12 ± 2	19·70, 19·68 (Mean, 19·69)	—	19·80, 19·85 (Mean, 19·83)
Extra dynamite II, ether extract—			
Nitroglycerin, 22 ± 1; trioil, 11 ± 1	21·90, 21·92 (Mean, 21·91)	—	22·02, 22·04 (Mean, 22·03)

* Diethyldiphenylurea.
† Dipentylphthalate.

The results in Tables I and II clearly indicate that different percentages of diphenylamine, dinitrotoluene, diethyldiphenylurea, dimethyldiphenylurea, dipentylphthalate and paraffin oil do not interfere in the quantitative determination of nitroglycerin present in admixture with these compounds by the Schultze - Tiemann method. Further, this method of analysis is simple and rapid, and gives accurate results.

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SHORT PAPERS

A Method for determining Orthophosphate in Water

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The methods currently recommended[1,2,3] for determining phosphate in water involve the reduction of molybdophosphoric acid, the blue colour so obtained being proportional to the amount of phosphorus present. In all of these methods close control of acidity is necessary, different acid concentrations giving rise to "blue" of various spectral properties.[4] Control of reaction temperature is important for reproducible results, and the colour formed is subject to fading. Stannous chloride is the reductant favoured when greatest sensitivity is required, but this reagent is unstable and must be freshly prepared. Variation in the amount of stannous chloride reagent used has also been found to have an effect on the final optical density produced by a known amount of phosphorus.

The vanadium phosphomolybdate method developed by Kitson and Mellon[5] appeared to be free from most of these disadvantages. A simpler version of their procedure, proposed by Hanson,[6] has recently been adopted as the official spectrophotometric method[7] for determining phosphoric acid in fertilisers and feeding stuffs. This method has now been investigated as applied to the determination of the phosphate content of water, and a rapid simple procedure has been evolved.

EXPERIMENTAL

Hanson's[6] procedure requires the addition of 25 ml of a vanadium molybdate reagent for 5 to 6·2 mg of phosphoric acid (as P_2O_5) in a final volume of 100 ml. Optical-density measurements of the resulting yellow colour are made at 420 mμ against a reference solution containing 5·0 mg of phosphoric acid similarly treated. The amounts of phosphate (as PO_4) to be found in water usually lie in the range 0 to 5 mg per litre. Hence, experiments have been performed to discover the minimum amount of vanadium molybdate to be used, the maximum permissible acid strength of the reagent and the wavelength for optical-density measurement best suited to the sensitivity required.

The proposed reagent contains one-half of the amounts of ammonium vanadate and ammonium molybdate suggested by Hanson.[6] One millilitre of this reagent is sufficient to determine up to 100 μg of phosphorus (as P). Because of the proposed decreased volume of reagent, it has been necessary to increase the nitric acid content somewhat. Further increase in acidity results in a decrease in sensitivity, but the method is insensitive to decrease in acidity owing to the alkalinity of most waters. To ensure the required sensitivity, optical-density measurements are made at 370 mμ in 4-cm cuvettes against distilled water. By using these reduced amounts of reagent the blank has been found to have the acceptable value of about 0·025. The reagent is stable for at least 3 months.

The rate of colour development is temperature dependent, but the maximum optical density is attained, in all reactions, within 10 minutes after adding the reagent. This maximum value is independent of temperature in the range 0° to 30° C and is stable for at least 2 hours.

Interference caused by the colour of some natural waters, whose absorption can be appreciable at 370 mμ, is avoided by determining the optical density of a second portion of the sample to which only dilute nitric acid has been added. Subtraction of this value from that obtained by the proposed procedure then gives a corrected figure related to the phosphate content of the water.

In a litre of water, up to at least 5000 mg of chloride, 20 mg of fluoride, 2000 mg of alkalinity (as calcium carbonate), 200 mg of silicate (as SiO_2) and 2 mg of ferric iron have been shown not to interfere in the proposed procedure. Polyphosphates (*e.g.*, hexametaphosphate and pyrophosphate) are not usually present in natural waters, but may occur in treated waters. They do not interfere in this method provided that the optical-density readings are taken not more than 30 minutes after adding the reagent. Polyphosphates are very slowly hydrolysed by the acidic reagent, the rate of hydrolysis appearing to increase with time.

The proposed method gives a reproducible and accurate determination of the soluble orthophosphate content (as PO_4) in the range 0·02 to 4 mg per litre of a water. The results obtained are similar to those obtained by the standard stannous chloride - molybdenum blue procedure, but they are achieved more simply and with greater certainty.

METHOD

REAGENTS—

All reagents should be of recognised analytical grade; solutions should be prepared in distilled or de-ionised water.

Vanadium molybdate solution—Dissolve 0·50 g of ammonium vanadate in warm water, cool, and add 125 ml of nitric acid, sp.gr. 1·42. Add a solution of 10·0 g of ammonium molybdate in water, and dilute to 1 litre.

Nitric acid, 2·0 N, *approximately.*

Standard phosphate solution—Dissolve 0·716 g of potassium dihydrogen phosphate (dried at 150° C) in water, and dilute to 1 litre. Dilute 20 ml to 1 litre as required.

1 ml ≡ 10 μg of phosphate as PO_4

PREPARATION OF STANDARD GRAPH—

Dilute to 50 ml appropriate volumes of the standard phosphate solution to the range 0 to 200 μg of phosphate as PO_4. To each add 1 ml of vanadium molybdate solution, mix well, and set aside at room temperature for 10 minutes. Measure the optical density of each test solution in 4 cm cuvettes against distilled water with a suitable spectrophotometer at 370 mμ. Plot a graph relating optical density to phosphate content; this should be linear.

PROCEDURE FOR COLOURLESS SAMPLES—

Take 50 ml of sample and proceed as described under "Preparation of Standard Graph" at ". . . add 1 ml of vanadium molybdate solution. . . ." Determine the orthophosphate content of the sample by referring the optical density so determined to the previously prepared standard graph.

PROCEDURE FOR COLOURED SAMPLES—

Proceed as described for colourless samples.

To 50 ml of sample add 1 ml of 2 N nitric acid, and determine the optical density of this solution as described above.

Determine the orthophosphate content of the sample by referring the difference in the optical densities obtained to the standard graph.

Turbid samples should be filtered, preferably through sintered-glass, before examination.

RESULTS

All optical-density measurements were made with a Unicam SP600 spectrophotometer.

RECOVERY OF ADDED PHOSPHATE—

The recovery of orthophosphate added to several waters of various sources and degrees of hardness has been determined by the proposed method; the results are shown in Table I.

TABLE I

RECOVERY OF ORTHOPHOSPHATE ADDED TO WATER

Source of water	Hardness as $CaCO_3$, mg per litre	Phosphate present, mg of PO_4 per litre	Phosphate added, mg of PO_4 per litre	Phosphate found, mg of PO_4 per litre
River	260	1·9	0·30	2·2
River	255	1·9	1·2	3·1
Borehole	290	0·15	0·60	0·72
River	330	0·85	3·0	3·9
Lake*	70	0·09	0·60	0·70

* This sample was coloured, Hazen 125.

REPRODUCIBILITY OF THE MEHOD—

The excellent reproducibility of the method is illustrated by the optical densities tabulated below obtained by repeated treatment of different waters.

Optical density due to water No. 1	0·532, 0·532, 0·532, 0·533, 0·532, 0·532
Optical density due to water No. 2	0·323, 0·322, 0·321, 0·322, 0·323

COMPARISON OF METHODS—

Determinations have been made by the proposed method and by a stannous chloride - molybdenum blue method[2] on several drinking and boiler-feed waters. The results obtained are shown in Table II.

TABLE II

COMPARISON OF METHODS

Results are expressed as mg of PO_4 per litre

Sample	Orthophosphate found by - Proposed method	Orthophosphate found by - Molybdenum blue method
Drinking water No. 1	0·93	0·95
Drinking water No. 2	0·01	Nil
Drinking water No. 3	0·55	0·55
Drinking water No. 4	0·12	0·12
Drinking water No. 5	0·09	0·12
Drinking water No. 6	1·9	1·9
Boiler-feed water No. 1*	0·21	0·19
Boiler-feed water No. 2*	0·15	0·16
Boiler-feed water No. 3*	0·36	0·34

* These samples also contained about 1·5 mg of sodium hexametaphosphate (as PO_4) per litre.

Permission to publish this paper has been given by the Government Chemist, Department of Scientific and Industrial Research.

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The Rapid Micro-determination of Carbon and Hydrogen with Magnesium Oxide-Catalyst Oxide Mixture

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THE use of magnesium oxide in slow combustion methods for determining carbon and hydrogen in the presence of fluorine has been advocated by several authors,[1,2] the tube filling consisting of separate layers of magnesium oxide and copper oxide. Campbell and Macdonald[3] first suggested the use of magnesium oxide on its own, especially for fluorinated materials, an oxygen flow rate of 12 ml per minute being maintained.

We have applied a single intimate mixture of magnesium oxide - catalyst oxide powders at temperatures between 900° to 1000° C and at an oxygen flow rate of 100 ml per minute with horizontal and vertical (Coleman) trains being used; the time required for combustion and sweeping is only 12 minutes. The proposed method has been tested on a wide range of organic compounds containing fluorine, chlorine, bromine, iodine, sulphur, nitrogen, phosphorus, arsenic, silicon, germanium and boron.

We have used mixtures of magnesium oxide with copper oxide, cobalt oxide, nickel oxide, vanadium pentoxide and platinum and have found the system magnesium oxide - copper oxide to be the best under the conditions described in this paper. Chlorine- and bromine-containing compounds were burnt with a mixture of magnesium oxide, copper oxide and silver.

Since most end-products of oxidative pyrolysis of organic compounds are acidic, basic conditions will accelerate the attainment of equilibrium, and this is most likely the function of magnesium oxide in the proposed mixture. When using copper oxide without magnesium oxide we obtained poor results under rapid conditions; conversely, magnesium oxide alone also proved inadequate, yielding low values for carbon.

The performance of the horizontal train was, in general, more uniform than that of the Coleman train.

METHOD

APPARATUS—

Horizontal train—The quartz tube (see Fig. 1) is 55 to 60 cm long and 11 mm in diameter. The rear portion of the tube (25 cm) is empty and serves for the insertion of the sample in a platinum boat; it is heated by a movable furnace of the split type, adjusted to approximately 950° C. The remaining portion of the tube is filled permanently and is heated by furnaces A and B. The lengths of the furnaces and the sequences of the layers surrounded by them are: A, 20 cm long, filled with granules of the magnesium oxide - catalyst oxide mixture; B, 7 cm long, filled with M.A.R. grade silver-wool, which occupies most of the gap between A and B. The "beak" of the combustion tube is packed with M.A.R. grade silver-wool. The temperatures of furnaces A and B are adjusted to 950° and 180° C, respectively.

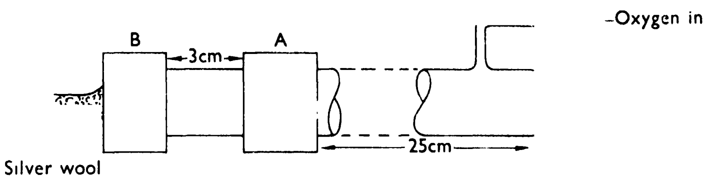

Fig. 1. Combustion tube and heating units

The pre-heater, pre-treat absorption tube, Rotameter, nitrogen oxide absorption tube and Flaschenträger-type absorption tubes are of conventional type.[4] The water-absorption tube is filled with M.A.R. grade anhydrone. The carbon dioxide absorption tube contains three layers: "carbabsorb" soda-lime (self-indicating granules, 15 to 20 mesh), ascarite and anhydrone. The use of ascarite alone may result in blocking of the carbon dioxide absorption tube under rapid conditions, and low values for carbon are obtained.

Vertical train, Coleman Model 33: *carbon - hydrogen analyser*—A detailed description of this automatic train is given in a pamphlet issued by the manufacturers.[5] We shall therefore restrict ourselves to listing the modifications introduced by us—

(*i*) The quartz combustion tube, which is 34·5 cm long and 10 mm in diameter, has an empty portion of 12·5 cm at the top end (for the insertion of the sample), this is followed by a layer of magnesium oxide - catalyst oxide mixture (12·5 cm) and finally by a roll of 40-mesh silver gauze (7·5 cm).

(*ii*) Carbon dioxide and pre-treat absorption tubes contain 15- to 20-mesh "carbabsorb" soda-lime.

(*iii*) The temperature of the final section of the metal tubing inside the apparatus is maintained at approximately 100° C. This eliminates fluctuations of the hydrogen values with changes in room temperature.

MAGNESIUM OXIDE - CATALYST OXIDE MIXTURE—

The materials may be of laboratory-reagent grade. The procedure described below was adopted for preparing the mixture. Equal amounts, by weight, of magnesium oxide and catalyst oxide powders* are cautiously mixed with water, until a thick uniformly coloured paste is obtained. After being dried at 120° C, the cake is ignited for 1 hour at 850° C in a muffle furnace.

* Copper oxide in wire form is also satisfactory, except for compounds that are difficult to burn, e.g., bispentafluorophenyl mercury, when copper oxide powder proved superior.

PROCEDURE—

In general, the procedure follows the pattern established for the rapid technique.[6,7] The platinum boat containing between 5 and 15 mg of sample is inserted in the combustion tube approximately 5 cm from furnace A, and the mouth of the combustion tube is closed. The operations listed in Table I require careful timing with a stopwatch.

TABLE I

DETAILS AND SEQUENCE OF OPERATIONS

Operation	Operating time, seconds	Flow rate, ml per minute	Position of furnace
Purge	50	100	Platinum boat halfway between furnace A and movable furnace
Pre-heat	120	0	Movable furnace pulled into position around the combustion tube
Slow combustion	120	4	Movable furnace brought up to the platinum boat
Fast combustion	120	100	Movable furnace brought up to furnace A
Final sweep	300	100	Position as for "Fast Combustion"

After the final sweeping, the absorption tubes are weighed within 2 minutes for the Coleman train and 3 minutes for the horizontal train. Thus, an over-all time for weighing plus determination is 16 minutes for the Coleman and 18 minutes for the horizontal train.

Some typical results for over 600 analyses are shown in Table II. With the horizontal train, about 200 analyses have been performed without the tube filling being changed.

TABLE II

TYPICAL RESULTS

Sample	Carbon found, %	Carbon calculated, %	Hydrogen found, %	Hydrogen calculated, %
Benzoic acid (M.A.S.)	68·92	68·84	5·05	4·95
Phenylthiourea (M.A.S.)	55·02	55·24	5·35	5·30
p-Chlorobenzoic acid (M.A.S.)	53·72	53·69	3·39	3·22
p-Bromobenzoic acid (M.A.S.)	41·70	41·82	2·62	2·51
o-Iodobenzoic acid (M.A.S.)	34·00	33·90	2·06	2·03
Trifluoroacetanilide (M.A.S.)	50·71	50·80	3·29	3·20
m-Trifluoromethylbenzoic acid (M.A.S.)	50·55	50·54	2·67	2·65
Biphenylsulphone	65·75	65·99	4·84	4·62
Triphenylphosphine (M.A.S.)	82·39	82·42	5·96	5·77
2-Aminoethoxydiphenylborane	74·8	74·7	7·4	7·1
sym-Tetraphenyldimethyldisilane	79·2	79·1	6·6	6·6
Manganese tricarbonyl-di-triphenylphosphine hydride	70·5	70·5	4·55	4·7
Phenyl manganese tetracarbonyltriphenylarsine	61·2	61·0	3·6	3·6
Hexaphenyldigermane	71·1	71·2	4·9	4·94

We thank Mr. P. Woodbridge for carrying out various analytical determinations.

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Book Reviews

RESIDUE REVIEWS: RESIDUES OF PESTICIDES AND OTHER FOREIGN CHEMICALS IN FOODS AND FEEDS. Volume 2. Edited by FRANCIS A. GUNTHER. Pp. iv + 156. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1963. Price DM 22.

The second volume of "Residue Reviews" is essentially similar in scope to the first (see *Analyst*, 1963, **88**, 486). All the chapters deal with pesticide residues and, with one exception, all are presented in English. Polarographic methods for the determination of insecticide and fungicide residues are reviewed, in French, by P. H. Martens and P. Nangniot (pp. 25); and, incidentally, this imparts a bilingual atmosphere to the subject index at the end of the volume The applicability of the method to copper, mercuric, arsenic, selenium and organo-tin compounds, to phosphides and to products containing elemental sulphur are briefly considered, followed by specific accounts of the use of polarography for nicotine, the pyrethrins and a full review of the work on various synthetic organic pesticide residue determinations in soils, seeds, fruit and vegetables.

The volume begins with a short account by A. L. Taylor (U.S. Department of Agriculture) on nematocide residues in plants (pp. 8, including three pages of summaries and references), about which there appears to be little published information. C. H. van Middelem writes on the nature, incidence and effects of parathion residues on leafy crops (pp. 22), the amount of published and unpublished information on which (he remarks) is exceeded only by that for DDT. This is a useful summary of the whole subject; chemistry, mode of action, toxicology, translocation and decomposition, residue determination and results, together with the implications of the latter in the United States. J. W. Mitchell and P. J. Linder write on the movements of plant growth regulating substances (pp. 26). The accumulation of residues is influenced by translocation and exudation mechanisms; by external factors such as those arising from the choice of wetting agent, dispersion solvent and the pH condition of the preparation applied; and other environmental factors.

Melvin E. Getz reviews published work on the determination of organophosphate pesticides and their residues by paper chromatography (pp. 17), emphasising the difficulties arising from the need to characterise not only the pure (or commercial) pesticides themselves, but also any toxic break-down products. The positive identification of individual constituent compounds still requires further attention. This is a straightforward review of published chromatographic systems, including clean-up methods and chromogenic agents. The longest chapter (pp. 54), by R. C. Blinn and the Editor, is on the use of infrared and ultraviolet spectrophotometric methods of residue analysis. This is a useful summary. It opens with a brief introduction to the theoretical basis for the spectra and the consideration of such practical details as the radiation sources and optical materials. Considerable use is made of tabulation in the presentation of the review of infrared work (5 Figs. and 7 Tables in all) and there are some hundred classified references to the use of the method in residue and formulation analysis and in metabolic studies. There is less to say of ultraviolet methods, but again a tabular summary of published methods for pesticide residues is provided. The authors remark, somewhat quaintly, that in the United States the field of residue chemistry has recently emerged from its gestation period; perhaps the same could be said of "Residue Reviews," although it remains to be seen what will be the eventual width of scope of the publication and how broad will be the shoulders of this lusty infant. H. EGAN

LINE INTERFERENCE IN EMISSION SPECTROGRAPHIC ANALYSIS: A GENERAL EMISSION SPECTROGRAPHIC METHOD INCLUDING SENSITIVITIES OF ANALYTICAL LINES AND INTERFERING LINES. By J. KROONEN and D. VADER. Pp. viii + 213. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price D.fl. 30; 60s.; DM 33.50.

This publication is the outcome of several years experience in the authors' laboratory with a general emission spectrographic method for the analysis of miscellaneous powdered samples. The sample is diluted with a lithium carbonate - graphite mixture, which acts as an internal standard and buffer, and this is excited in a d.c. arc.

The authors have studied the characteristics of 38 of the more common elements and most of the book (190 pages, in fact) is taken up with tables giving data on their analytical lines. For each analytical line is given its wavelength, excitation potential (where known), sensitivity, useful concentration range and a list of interfering lines within the interval ±0·65 Å. The sensitivities of interfering lines and differences in wavelength from the analytical line are also included. Over

500 lines are listed with about 8500 interfering lines, of which nearly 1000 are recorded for the first time. Full details of the method used are given in the early pages of the book.

Two basic shortcomings are almost inevitable in any book of this kind: the incomplete coverage of the elements that are detectable by emission spectrography, and the fact that the information it contains does not necessarily apply when alternative methods of excitation are used. For instance, no information is given on any of the metals of the rare-earth or platinum groups (with the exception of platinum itself). The sensitivities quoted both for analytical and interfering lines apply only when the recommended method, or a method giving a similar arc temperature (about 5500° K), is used. For example, the authors recognise that their tabulated lists of sensitivities are different from those published by the N.B.S. based on a copper arc.

In laboratories where semi-quantitative spectrographic methods differ from those described, little value will be gained from this book, because the recorded sensitivities are not applicable and most of the interfering lines will be found in the more extensive M.I.T. wavelength tables.

When, however, the recommended method is, or can be, used and particularly where the development of a general spectrographic method of analysis is intended, the book will be invaluable.

The size of the book (12 inches × 8½ inches) is rather large for many book cases, but the layout and the printing make the tables easy to read. J. A. F. GIDLEY

MICROCHEMICAL TECHNIQUES. Edited by NICHOLAS D. CHERONIS. Microchemical Journal Symposium Series. Volume II. Pp. xviii + 1181. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1962. Price £15.

This book contains the proceedings of the 1961 International Symposium on Microchemical Techniques organised by the Metropolitan Microchemical Society at the Pennsylvania State University, U.S.A. The contents are divided into Introduction (Welcoming Addresses and 2 "historical" papers), Plenary Lectures (4), Microscopical Techniques (10 papers), Techniques of Inorganic Elemental Analysis (7 papers), Techniques of Organic Elemental Analysis (17 papers), Techniques of General Organic and Functional Group Analysis (8 papers), Chromatographic Techniques (8 papers), Titrimetric, Polarographic, Electrochemical and Radiochemical Techniques (19 papers), Microchemical Implements and Techniques for the Determination of Molecular Weight and Physical Properties (13 papers), Micro and Semimicro Methods in Teaching (6 papers) and Round Table Discussions (5 topics). The discussion is reported for several of the papers; an author and subject index complete the book.

The Section of Analytical Chemistry of the International Union of Pure and Applied Chemistry extended its sponsorship to the Symposium, a clear indication of the high quality of the papers. The book itself maintains the standard of production set by Volume I of the Series (see *Analyst*, 1962, **87**, 415). It is a credit both to the publisher and to the editor, whose unfortunate death occurred before the publication was completed. Although the price places it well outside the pocket of the individual purchaser, the book seems certain to become a valuable library reference work.

A point of some concern to myself, and no doubt to others, is the time lag that often elapses before symposium proceedings are finally published. In the present instance, I was particularly interested in some of the papers in mid 1962, by which time only the paging of the text had been reached. Although it is virtually impossible to get all of some 100 authors to submit their manuscripts by any agreed date and the volume of editorial work is large, surely something can be done to hasten publication. One possible solution is that successfully adopted in connection with the International Gas Chromatographic Symposium held at Houston, Texas, U.S.A.; in January, 1963. It was stipulated that all of the papers must deal with original material and be submitted to *Analytical Chemistry* for review before the meeting. The few papers not meeting the deadline were reviewed during the symposium itself. In this way it was possible to publish the 23 symposium papers in the April issue of *Analytical Chemistry*. M. WILLIAMS

ROCK-FORMING MINERALS. Volume 4. FRAMEWORK SILICATES. By W. A. DEER, M.Sc., Ph.D., F.R.S., R. A. HOWIE, M.A., Ph.D., F.G.S., and J. ZUSSMAN, M.A., Ph.D., F.Inst.P. Pp. x + 435. London: Longmans, Green & Co. Ltd., 1963. Price 95s.

The publication of Volume 4 sees the completion of this work on the rock-forming minerals, selected as they are on the basis of their presence or absence determining or modifying the name of a rock.

This volume deals with the so-called framework silicates, *viz.*, the felspars, the zeolites, the silica minerals quartz, tridymite, and cristobalite, the sodalite and nepheline groups, and the single minerals petalite, leucite, cancrinite, scapolite and analcite, on the lines used in the volumes published earlier (see *Analyst*, 1963 **88**, 246).

The 1620 chemical analyses spread throughout the five volumes are the main feature for the analyst; but there is, besides these plenty that is of wider chemical interest in this work, which presents an up-to-date account of modern views on the many important minerals that have been selected for consideration.

The printing and the diagrams are well done, as, indeed, they should be at the price charged for each volume.

L. S. THEOBALD

PROBLEMS IN INORGANIC CHEMISTRY. By HOWARD NECHAMKIN. Pp. x + 171. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Company Inc. 1963. Price 30s.

The problems set and illustrated in this book are designed to illuminate the general range of topics covered by an up-to-date course on Inorganic Chemistry and they should help the advanced student to a fuller understanding of his subject. Among analysts, only those who have some knowledge of modern ideas on inorganic chemistry are likely to be interested.

L. S. THEOBALD

Publications Received

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MASS SPECTRAL CORRELATIONS. By FRED W. MCLAFFERTY. Pp. vi + 117. Washington, D.C.: American Chemical Society. 1963. Price $4.75.
Advances in Chemistry Series No. 40.

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PRINCIPLES OF CATALYSIS. By G. C. BOND, B.Sc., Ph.D., F.R.I.C. Pp. vi + 51. London: The Royal Institute of Chemistry. 1963. Price 6s.
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IDENTIFICATION OF ORGANIC COMPOUNDS: A STUDENT'S TEXT USING SEMIMICRO TECHNIQUES. By NICHOLAS D. CHERONIS and JOHN B. ENTRIKIN. Pp. xii + 477. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 65s.

PARTICLE SIZE: MEASUREMENT, INTERPRETATION AND APPLICATION. By RIYAD R. IRANI and CLAYTON F. CALLIS. Pp. x + 165. New York and London: John Wiley & Sons. 1963. Price 60s.

ADVANCES IN X-RAY ANALYSIS. Volume 6. Edited by WILLIAM M. MUELLER and MARIE FAY. Pp. xii + 480. New York: Plenum Press Inc. 1963. Price $17.50.
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MASS AND ABUNDANCE TABLES FOR USE IN MASS SPECTROMETRY. By J. H. BEYNON and A. E. WILLIAMS. Pp. xxii + 570. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 80s.

TOPICS IN MODERN CHEMISTRY. By C. J. MANDLEBERG, M.A., B.Sc., F.R.I.C. Pp. xii + 228. London: Cleaver-Hume Press Ltd. 1963. Price 37s. 6d.

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Reprints of Review Papers

"Beer's Law and its Use in Analysis"

REPRINTS of the Review Paper, "Beer's Law and its Use in Analysis," by G. F. Lothian, published in the September, 1963, issue of *The Analyst* (pp. 678–687), are available from the Secretary, Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free.

"A Review of the Methods Available for the Detection and Determination of Small Amounts of Cyanide"

REPRINTS of the Review Paper, "A Review of the Methods Available for the Detection and Determination of Small Amounts of Cyanide," by L. S. Bark and H. G. Higson, published in this issue of *The Analyst,* will soon be available from the Secretary, Society for Analytical Chemistry, at the above address, price 5s. per copy, post free.

A remittance for the correct amount, payable to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not available through Trade Agents.

Erratum

JULY (1963) ISSUE p. 540 line 3. For "0·1-mm cells" read "1·0-mm cells".

NOVEMBER, 1963 Vol. 88, No. 1052

THE ANALYST

EDITORIAL

Further Development of *The Analyst*

SIX months ago the Society introduced a revised "Notice to Authors" and, simultaneously, a modified system for subjecting papers to the scrutiny of referees. At the same time we mentioned in an Editorial some alterations in the appearance of *The Analyst* that had taken place at the beginning of the year. Then, after outlining the changed responsibilities of the Editorial (formerly Publication) Committee, and the formation of the Publications Policy Committee, we said that many proposals of the *Analyst* Development Committee remained to be implemented. These changes (like the change in the paper stock on which the journal is printed) could not be introduced except at the beginning of a volume, but will take place in January, 1964.

The most important of these proposals was undoubtedly one that the Proceedings of the Society, *i.e.*, the reports of the activities of the Society, its Sections and its Groups, other "domestic" news pertaining to the Society, obituaries, and certain other items, should be published separately from the scientific matter at present making up most of *The Analyst.* The Publications Policy Committee has given further consideration to this proposal and has studied possible alternatives and matters of detail. Its report has been accepted by Council, which has decided that from January *The Analyst* shall be devoted entirely to matters appropriate to the journal of a learned Society. It will contain Special Lectures, Review Papers, Original Papers (including Short Papers), Book Reviews, Communications and, when required, Editorials.

Communications are a new feature being introduced in response to requests for a medium for the prompt publication of urgent material. It is not intended for simple claims to priority, but rather for the brief description of work that has made some progress and then has stopped for lack of time, or of resources, or even of inspiration. Publication has a twofold purpose: the ideas, although not fully worked out, may yet be valuable to workers on other problems, or other workers may be able to provide the necessary inspiration or resources to permit the completion of the original piece of research.

Certain conditions must be fulfilled. Manuscripts must obviously be on urgent matters of some scientific importance (for time does not permit examination by referees), and must not exceed 300 words. They cannot include diagrams, although there will be no objection to formulae or tabular matter. Timing also precludes any question of appeal against the Editor's refusal to include a particular Communication, although a manuscript so refused can always be submitted to the Editorial Committee for its decision after the usual examination by referees. In return *The Analyst* offers publication in a minimum time from receipt of 4 to 5 weeks, which is the length of the final stage of the production schedule. To this it may be necessary to add 30 days should a script arrive 1 day too late for a particular issue, making the maximum time 9 weeks. Such rapid publication can only be achieved for a small proportion of the material in each issue.

The *Proceedings of the Society for Analytical Chemistry* will bring the rest of the traditional contents of *The Analyst* to members and non-members alike, and will also incorporate notices of forthcoming meetings and other material that has hitherto appeared in the *Bulletin*. The Publications Policy Committee foresees the possibility of the *Proceedings* developing as a vehicle for other material worthy of the widest possible dissemination. *Proceedings* will therefore be treated from its inception as a fully-fledged journal and will be supplied in due time with a title page and index to each volume to facilitate the binding of this record of the Society's activities.

Two other changes remain to be mentioned. The cover of *The Analyst* has been re-designed slightly to lay greater emphasis on the fact that it is the journal of our Society. Also, in response to several requests, space will be found among the advertisements for duplicate copies of summaries of papers in a form in which they can readily be cut out for pasting on index cards, a service already available in other major analytical journals.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETINGS

An Ordinary Meeting of the Society was held at 6.30 p.m. on Wednesday, October 9th, 1963, in the Main Chemistry Lecture Theatre, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7. The Chair was taken by the President, Dr. D. C. Garratt, Hon.M.P.S., F.R.I.C.

The subject of the meeting was "Thin-layer Chromatography" and the following papers were presented and discussed: "General Aspects," by L. J. Morris, B.Sc., Ph.D.; "Specific Separations on Impregnated Thin Layers," by L. J. Morris, B.Sc., Ph.D.; "Application of Thin-layer Chromatography to Inorganic Systems," by K. Burton, B.Sc., D. Lyons, B.Sc., G. Nickless, B.Sc., Ph.D., and F. H. Pollard, D.Sc., Ph.D.; "Effects of Alkyl and Alkenyl Substitution on the Chromatography of Quinols and Related Compounds," by D. McHale, B.Sc., Ph.D., A.R.I.C.; "Separation of Steroids on Microslides," by P. Oxley, M.A., B.Sc., A.R.I.C.; "Thin-layer Chromatography of Lipids," by B. W. Nichols, M.Sc., Ph.D.

DEATHS

We record with regret the deaths of

Leonard Balmforth
Daryl Robert O'Dea
William Stross.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, October 15th, 1963, at the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by the Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Sugar in Foodstuffs—Some Newer Methods," by J. L. Buchan, M.Sc., A.R.I.C.

MICROCHEMISTRY GROUP

The forty-first London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 16th, 1963, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Mr. T. R. F. W. Fennell, B.A.

A discussion on "The Determination of Boron" was opened by H. J. Cluley, M.Sc., Ph.D., F.R.I.C., and M. R. Hayes, A.R.I.C.

Circular Dichroism

A Review*

BY R. D. GILLARD

(Department of Chemistry, Imperial College of Science and Technology, London, S.W.7)

Plane-polarised light may be resolved into *laevo-* and *dextro*-circularly polarised light. When it is passed through an optically active medium, the amplitudes of these components are initially equal, but if the refractive indices of the medium are different for each component, there is rotation of the emergent light, which is now elliptically polarised. The phenomenon of circular dichroism (the differential absorption of *laevo-* and *dextro*-circularly polarised light) is a function of wavelength and the shape of the molecules in the medium.

This short review draws attention to a technique that may well prove invaluable in analysis.

INTEREST in optical activity has recently revived.[1] The most commonly used property of optically active molecules in research applications has been the optical rotatory dispersion curve (the variation of specific rotation of plane polarised light with the wavelength of the light). Several valuable reviews of this technique are available, for example, those of Djerassi[2] and Klyne,[3] but so far it has not attracted attention as an analytical method, probably because the rotatory dispersion curves of single substances are not easy to interpret, and it is difficult, although not impossible, to draw quantitative conclusions from the rotatory dispersion curves of mixtures.

Plane polarised light may be resolved into two circularly polarised components with equal amplitudes. Optical rotatory power is attributed to a difference in refractive indices of the optically active material for *laevo* (left) and *dextro* (right) circularly polarised light. If η_l (the refractive index for left circularly polarised light) is larger than η_d (that for right circularly polarised light), the left circularly polarised light is delayed in traversing the medium, giving rise to a rotation of the plane-polarised resultant of the left and right circular components. In passing through absorption frequencies of an optically active molecule, the absorption of the left-handed component differs from that of the right-handed component. With the usual notation of ϵ for molar extinction coefficient and with appropriate suffixes, circular dichroism at any wavelength is given by $\epsilon_l - \epsilon_d$, which we call $\Delta\epsilon$. Cotton[4] observed this in solutions of the tartrates of transition metals, and the phenomenon became known as the Cotton effect.[5] Since circular dichroism arises through electronic transitions, the spectra produced are similar to ultraviolet and visible spectra, but, for each circular dichroism band, $\Delta\epsilon$, ($\epsilon_l - \epsilon_d$), may be either positive or negative, depending on the handedness of the molecule concerned. The requirements for the observation of a circular dichroism band are seen to be an optically active (asymmetric) molecule and an electronic absorption band. Any chromophore will give rise to circular dichroism bands; an example is the carbonyl ($>C=O$) absorption ($n \rightarrow \pi^*$) at 3000 Å. The circular dichroism of this band for a 16-ketosteroid is shown in Fig. 1.

MEASUREMENT

The most obvious method of obtaining circular dichroism, $\Delta\epsilon$, is to measure the difference in optical density for *laevo-* and *dextro*-circularly polarised light; apparatus has been described to permit this to be done.[6] However, the method generally used involves the fact that, after plane polarised light has passed through an optically active medium *in a region of electronic absorption,* the circular components have been unequally absorbed, so that on recombination of the unequal components, elliptically polarised light is obtained instead of plane, polarised light. As the ellipticity is a function of the difference in absorption of *laevo-* and *dextro*-circularly polarised light, it is directly related to circular dichroism. The molecular ellipticity is given by—

$$[\theta] = 3300\ \Delta\epsilon.$$

Most measurements of circular dichroism have been based on ellipticity determinations, as noted by Mitchell.[7] A recent commercial instrument, in which an oscillating crystal technique[8] is used, is also based on this well tried principle.

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* **Reprints of this paper will be available shortly. For details, please see p. 902.**

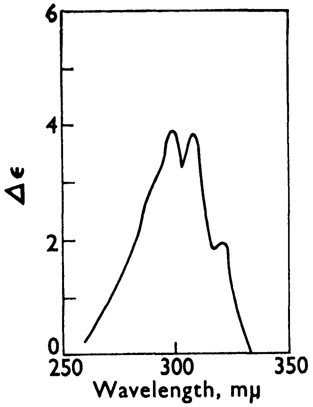

Fig. 1. Circular dichroism for ($n \rightarrow \pi^*$) band of carbonyl group in a 16-ketosteroid

CHEMICAL APPLICATIONS

The principles implicit in the previous discussion may be summarised as:

(*a*) Circular dichroism is a function of wavelength, λ, with positions of maxima coincident with visible or ultraviolet absorption bands. However, two bands that are not resolved in the ordinary electronic absorption spectrum are often separate in circular dichroism. The selection rules for circular dichroism and electronic absorption are often different, which leads to useful differences in band shapes.

(*b*) The sign of a circular dichroism peak depends on the actual shape ("handedness" or "absolute configuration") of the molecule causing it.

So far, circular dichroism has been studied in very few systems. Its uses seem likely to be in the fields detailed below:

(*i*) Analytical studies of mixtures, either from reactions or from natural sources. This will be discussed later.

(*ii*) Relative and absolute configurations of natural products such as steroids, terpenes, proteins and sugars. For example, the absolute configuration of the diterpenoid, cafestol, has been shown[9] to be the opposite to that previously deduced from optical rotatory dispersion. Several other erroneous configurations based on optical rotatory dispersion have also been corrected[9] by studies of circular dichroism.

(*iii*) Determination of structural features in complex organic molecules. It has been found[10] that the circular dichroism of the 20-cyano-derivatives of 11-ketosteroids permitted the isomers to be distinguished and formulated, whereas neither the nuclear magnetic resonance nor infrared spectra were sufficiently distinctive to be useful. Similar applications are feasible in inorganic complex compounds. Since more circular dichroism bands appeared for the (—)trisoxalatocobaltate$^{\text{III}}$ anion, (—)[Co(C_2O_4)$_3$]$^{3-}$, than are expected for an ion with D_3 symmetry, the presence of some aquated ions of lower (C_2) symmetry was postulated,[11] with the formula [Co(C_2O_4)$_2$(C_2O_4H) (OH_2)]$^{2-}$.

(*iv*) Relative and absolute configurations of octahedral co-ordination compounds. It has been deduced from the equivalence of the circular dichroism curves[12] for the (—)$_{546\cdot 1}$[Co(EDTA)]$^-$ and the (—$_{546\cdot 1}$[Co(+PDTA)]$^-$ ions (where EDTA is ethylenediaminetetra-acetate and +PDTA is dextrorotatory propylenediaminetetra-acetate), that their relative configurations are the same. The absolute configuration of (—)$_{546\cdot 1}$[Co-(+PDTA)]$^-$ is known (Fig. 2), so that the absolute configuration of (—)$_{546\cdot 1}$[Co(EDTA)]$^-$ is seen to be the same.

(*v*) Because of the differences in selection rules for circular dichroism compared to electronic spectra (magnetic-dipole-allowed transitions appear strongly in circular dichroism), distinctions may be made between spectroscopic bands. Confirmation was obtained in this way of the assignment[13] of the lowest energy transition ($^1T_1 \rightarrow {}^1A_1$) in octahedral cobalt^{III} complexes. It was noticed in studies of optical rotatory dispersion that 2-α-iodocholestan-3-one gave rise to a strong Cotton effect centred at about 300 mμ, whereas no ultraviolet absorption band was observed in this region. Re-examination of the compound by Gillard (unpublished work) and Bose and co-workers[14] showed that the ultraviolet band at 298 mμ had an ϵ_{max} value of less than 4, whereas the circular dichroism band had a value for ($\epsilon_l - \epsilon_d$) of about 1, so that the magnetic dipole character of the transition responsible for the circular dichroism band is beyond doubt.

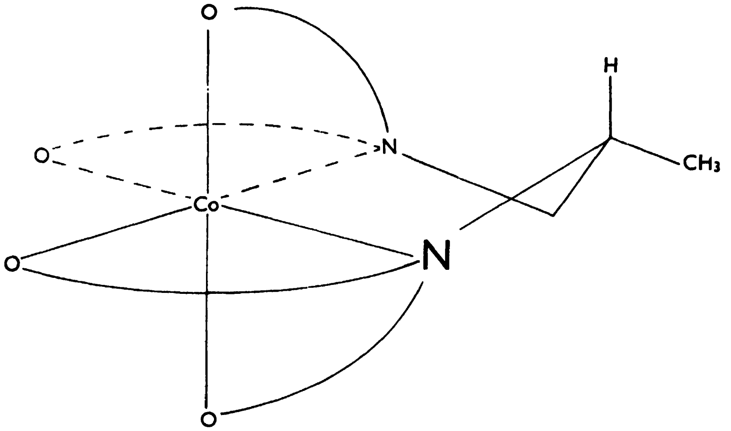

Fig. 2. The absolute configuration of $(-)_{546 \cdot 1}[Co(l\text{-}PDTA)]^-$ (each curved chelate ring represents - CH_2COO)

Analytical uses

Wherever optical activity occurs, as in nearly all natural products, circular dichroism is an obvious means of measuring both chemical and optical purity, since circular dichroism is a function of the concentration of the optically active species giving rise to it. For example, in the carbohydrate field, pure methyl-2-oxo-3,4-isopropylidene-β-L-arabinoside shows a circular dichroism maximum of $\Delta\epsilon = 1{\cdot}8$ at 312 mμ. However, as in any spectrophotometric method, circular dichroism is useful only for chromophores absorbing between about 2000 and 7000 Å. Concentrations required are about the same as those used for other spectrophotometric work, and the results are accurate to approximately 1 per cent. The amount of an optically active substance in an otherwise inactive mixture may be determined; applications to such materials as pine oil distillates and pyrethrum products are obvious.

The occurence of the circular dichroism of a particular chromophore, such as carbonyl or nitro groups, within a narrow range of wavelengths offers possibilities for a "fingerprint" method of qualitative analysis for functional groups, particularly in synthetic products. So far, the characteristic circular dichroism of only a few functional groups is known, but more examples are being added rapidly. One might expect at least the chromophores listed below to find application: for α-amino-acids, the *N*-phthaloyl[15] and thiourea[16] derivatives; for cyclo-alkenes, the osmate esters[17]; for α-substituted carboxylic acids, the acylthiourea derivatives[18]; and for α-hydroxyacids the dithiocarbonate derivatives.[16] Other chromophores that have been used in optical rotatory dispersion include thiones,[19] disulphides,[20] *N*-nitrosoamides[21] and nitrites.[22] (References are to optical rotatory dispersion work.)

The cobalamin system presents many analytical problems; an interesting recent study[23] presents the circular dichroism curves for several cobalamins. These curves show much more marked differences than the absorption spectra; for example, for cyanocobalamin and aquocobalamin, which have similar absorption spectra, the circular dichroism bands at 500 mμ are, respectively, negative and positive, and the signs of several other bands are also different for the two compounds.

CONCLUSION

Little analytical work involving the use of circular dichroism has appeared to date, though mixtures of 11-ketosteroids and 20-ketosteroids have been analysed by circular dichroism.[24] However, circular dichroism has many potential applications, particularly in the field of natural products, for which the technique may prove useful for handling mixtures now amenable only to complicated treatments.

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Received *July 9th*, 1963

Information Retrieval in the Analytical Laboratory

A Review*

By D. R. CURRY

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The potential applications of modern information-retrieval techniques in the analytical laboratory are outlined. A summary is given of the simple manual punched-card systems available. Attention is drawn to published material on the various aspects of the subject.

EVERY analytical laboratory deals with information, both as a receiver and as a creator. The process of analysis gives a continuing stream of new information about samples being examined. It is desirable that this information should be stored accessibly so that the effort originally expended in getting the analytical result is not wasted. At the same time the analytical chemist has, as the starting material for many of his investigations, the ever increasing scientific literature. In both these aspects the techniques of information retrieval may be useful. Information retrieval in this context is taken to mean manual systems as opposed to automatic data-processing techniques requiring the use of electronic computers. Save in exceptional circumstances the use of electromechanical card-sorters is seldom justified. They achieve little that cannot be done manually and may frequently take longer. In this paper the requirements of information retrieval are discussed under three headings—

(*a*) Laboratory records of work done;
(*b*) Reference data for instrumental methods;
(*c*) Literature.

As the analytical chemist is personally concerned in these problems, it is most strongly urged that study of the techniques available will prove worthwhile. Purely office routines are properly the concern of an O. and M. Officer, but manipulation of scientific facts should not be delegated to unqualified personnel.

THE PROBLEM OF LABORATORY RECORDS—

Most analytical laboratories, whether routine or research, have some method of recording samples, in which a costing procedure may also be incorporated. The system used should be able to provide the head of the laboratory with up-to-date information on the work in hand. At the same time records of past work done should not be left to memory, since the staff may change or retire. It should give all relevant details on previous examinations of particular types of sample; and also, for administrative purposes, show the numbers of these types of sample being analysed (*i.e.* how the flow of work is varying).

INSTRUMENTAL METHODS AND LITERATURE—

In many instrumental methods the recorded characteristics of a sample are assessed by comparison with similar records of known materials. At the same time the collection of standard records should be accessible via the various materials or classes of materials. In this way one should be able to find the characteristics that a series of organic homologues may have in common.

Many analytical laboratories are only small parts of larger organisations and their libraries may not, either through limitations of staff or knowledge of subject, be able to provide the analytical chemist with an adequate bibliographic service. Analytical information may not be found exclusively in the primary analytical literature or in abstracts, as some relevant material is bound to appear in the general literature of the field in which the analyst is working, *e.g.* petroleum, metallurgy or semi-conductors. The complexities of cross referencing often dissuade the analytical chemist from maintaining his own conventional card index. At the same time the analytical chemist may partly remember the reference of a particular item of interest (*e.g.*, author or journal) and an index should also answer problems from this approach.

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* Reprints of this paper will be available shortly. For details, please see p. 902

In all the instances mentioned above, records and information accessible in the laboratory and under the control of chemical staff are needed. Before considering detailed methods for tackling these problems, a brief outline is given of the various types of manual punched cards.

MANUAL PUNCHED CARDS

EDGE-PUNCHED CARDS—

Edge-punched cards are the simplest of manual punched cards, and their form is familiar to most people.[1 to 8] The cards have a series of holes along the edges, so that a slot may be formed by punching away the small amount of card between the hole and the edge. When a pack of cards is aligned and a sorting needle inserted through one of the holes, those cards that have been slotted in that particular position will fall away. The holes may be assigned various meanings, and a given set or "field" of holes grouped together to represent letters or figures. It is also possible to have more than one row of holes along part of the edge, to increase the number of selections available. The cards may be sorted by hand as indicated or in some systems by simple frames holding a series of needles, so that all the slots in one edge of a series of cards can be sorted simultaneously. Systems have also been devised for punching plain cards and sorting them on an array of bars.[9]

BODY-PUNCHED CARDS—

The theory of body-punched cards is the same as that of edge-punch cards. Instead of slots at the edge, however, slots are punched to join pairs of holes in the body of the card. Sorting has to be carried out in a special cradle that permits all the slots to be examined simultaneously. There is an unpunched area on the card for written information.[10]

FEATURE CARDS—

Feature cards are known by various names—coincidence cards, Batten cards or more popularly "Peek-a-boo" cards. The principle on which these operate is the reverse of edge punched item cards and is illustrated in Fig. 1. A feature card is prepared for each feature or concept considered relevant to part of a collection of items (such as abstracts). Each item is assigned a serial number, and every feature card has a number of squares, each corresponding to a particular numbered item. When a feature corresponds to a particular item a hole is punched in that item's square in the relevant feature card. When a series of feature cards is superimposed, any co-incident holes represent items possessing all the features selected. Feature cards are punched for items, whereas edge-punched item cards are slotted for features.

COMPARISON OF ABOVE SYSTEMS—

For a small collection of items not requiring complex indexing, the edge-punched cards offer economy and convenience. They have an important use, since they also reveal any concurrence of features. For example, if a series of cards is selected from a collection by sorting for holes A, C and D, and all the selected cards are also slotted in position B, this is immediately obvious as another common feature.

Feature cards are most valuable when a higher degree of cross referencing of a large number of features is essential. Speed of sorting is greater than with either of the other two systems, provided that the number of items sought is small, as the items themselves have to be in a subsidiary numerical file. The feature-card system is most adaptable to changes of emphasis as new subjects of interest develop. It is usual with edge- or body-punched cards to use custom-printed cards, so that the user does not have to refer to a book for the meanings of the various holes. Although unused holes should be available in the original design of the card their use will be limited, whereas it is a simple matter to introduce new feature cards at any time.

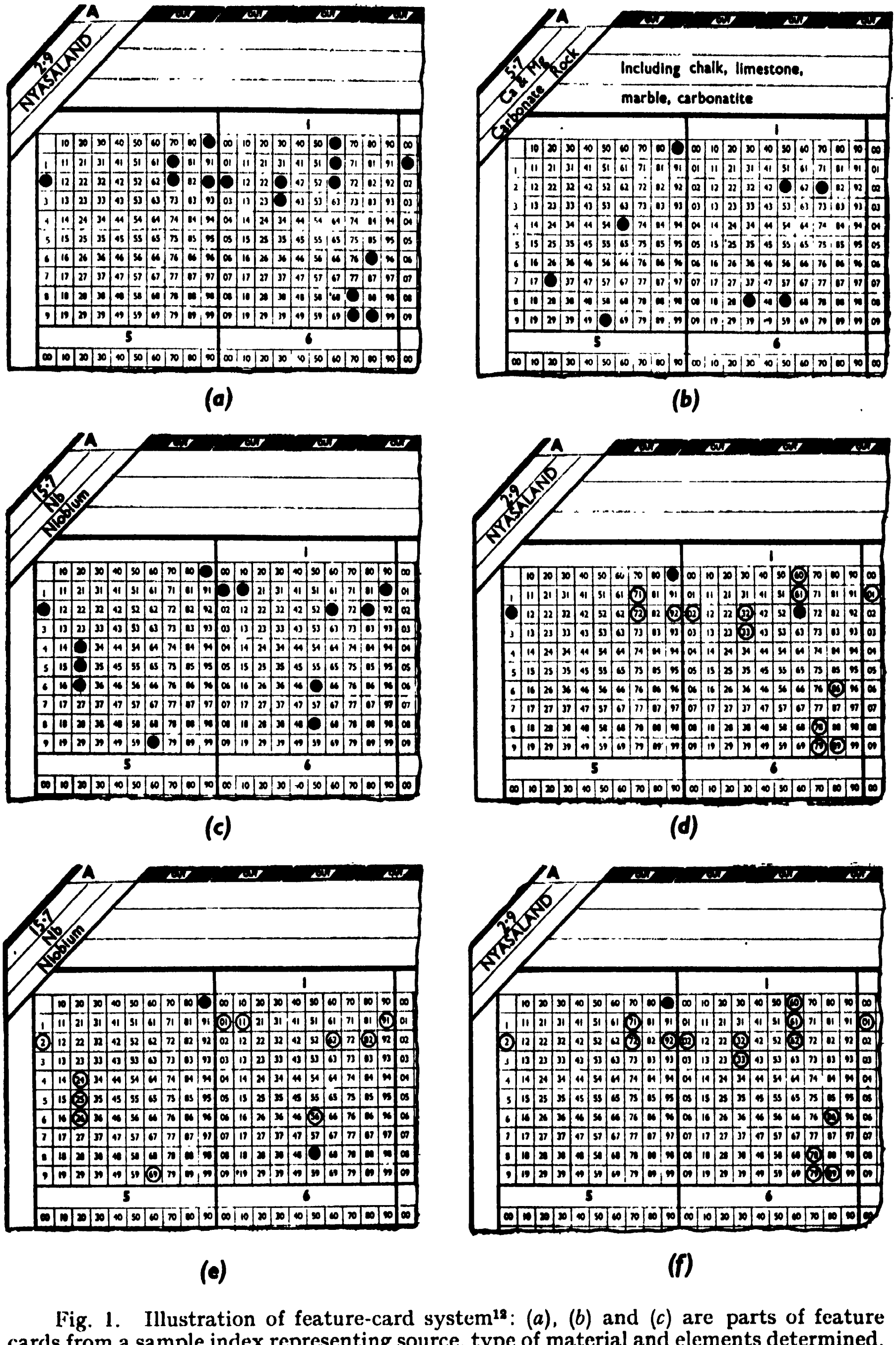

Fig. 1. Illustration of feature-card system[12]: (*a*), (*b*) and (*c*) are parts of feature cards from a sample index representing source, type of material and elements determined, respectively. Each hole in a card represents the numbers of samples having that particular feature. (*d*) shows the position when card (*a*) is superimposed on card (*c*) (*i.e.*, samples 2, 90 and 162 originated from Nyasaland and were analysed for niobium). Similarly (*e*) illustrates card (*c*) when placed on top of card (*b*), showing 90 and 158 as common holes. If all the cards are put together the result is shown by (*f*), only sample 90 being a carbonate rock from Nyasaland analysed for niobium. (Published by permission of the Controller, H.M. Stationery Office)

LABORATORY RECORDS

Although there are various references to efficient laboratory organisation, for example see Kent-Jones,[11] little literature giving practical details of achieving such control has been published. One the earliest comprehensive systems used an edge-punched master card for each sample analysed[1] and for a routine laboratory, a simple system has been advocated.[2] In the United Kingdom one of the edge-punched cards in use for some years at the Macaulay Institute for Soil Research, Aberdeen, has been discussed.[3] There is an example of the use of body-punched cards for record keeping (although not specifically chemical),[10] and recently a machine has been designed[9] at the Royal Armament Research and Development Establishment, Fort Halstead, Sevenoaks, Kent for converting plain record cards for edge-notched sorting. The application of feature cards to laboratory records has also been reported.[12]

REFERENCE DATA FOR INSTRUMENTAL METHODS

Before considering reference data in detail, it should be remembered that the quality of data retrieved can only be as good as the input. All authors, referees and editors of primary literature presenting data should give attention to this matter, which has been discussed by the Director of the Office of Critical Tables.[13]

X-RAY DIFFRACTION—

Chronologically, the first reference data system available on a commercial basis was the "X-Ray Diffraction Data Index" published in 1942 by the American Society for Testing Materials. In this system the three strongest lines in terms of inter-planar spacing of the powder diffraction pattern are used to select a card giving the full details of the pattern and the reference to the source of information. When the series was initiated, plain cards were used and filed numerically, but with the use of edge-punched cards, advocated by Matthews,[4] the three strongest lines and the chemical composition could be indexed. Practical use, however, established that the cumulative index book was most efficient, except for special searches meriting the use of I.B.M. cards. More recently, a proposal by Dr. Matthews to use a feature card index has been adopted.[14] Whereas the edge-punched cards were supplied neither punched nor marked for punching, the set of 150 feature cards covering 5,698 substances are supplied punched, and it is stated that "as new sections are added to the data file the feature cards can be returned for the additional data to be entered." The 10,000 position feature cards should therefore facilitate searching the index for identification purposes.

INFRARED SPECTRA—

In the United Kingdom there are two common systems for indexing infrared spectra. The Sadtler[15] system is in book form and has various indexes covering formula, name of compound and also the "Spec-finder" for identification of unknown spectra. In this procedure the strongest bands in twelve sections of an unknown spectrum are listed and compared with a numerical list automatically prepared by computer. A separate section of the Sadtler index deals with commercial products.

The Butterworth-D.M.S.[16] system was described by Thompson[5] and involves the use of edge-punched cards, which are available punched or marked ready for punching. The strongest bands in the spectrum are indexed and also various structural features of the compound. The body of the card also gives bibliographic details and physical properties in addition to the full spectrum. In 1960 a second series of cards was started, in which the same indexing procedures for inorganic spectra was used. Concurrently with the spectral cards, a series of literature abstract cards is supplied; these are similar edge-punched cards indexing author or authors, year of publication and the topics covered (theories, apparatus, etc.). Recently, a feature-card index to the spectral series has been issued (DMS-I-Cards); 211 of these deal with an extended range of the holes in the original series, and the capacity of each card is 5000 items.

A valuable collective index[17] of available spectra has recently been compiled and will be kept up to date by annual supplements.

DIFFERENTIAL THERMAL ANALYSIS—

The latest recruit to punched card data indexes is the Scifax DTA data index issued in 1962.[18] This is based on a simple edge-punched card, the body of which contains details of the differential thermal analysis record and bibliographic references. One edge is supplied punched with principal peaks, the remaining edges being left free for the user to punch as required, although for minerals it is suggested that the Hey classification be used.

LITERATURE SYSTEMS

The universal decimal classification for analytical chemistry,[19] class 543, may serve its purpose for arranging books, but a single glance should reveal its inadequacy for indexing complex papers in the literature. The problems associated with comprehensive indexing of analytical literature have received more attention than other aspects of information retrieval previously considered. Three papers on edge punched cards[6,7,8] give various examples of the different ways information can be coded by using numbers and letters. It is possible to index authors' names, substances, subjects and dates, thus permitting a reasonable amount of cross referencing.

Recently in American literature a co-ordinate indexing system has been introduced.[20] This is based on the feature-card principle, although for economy the method suggested is the 'Uniterm' card system. In my opinion this is a false economy, since the manual effort of entering numbers on cards and recognising correlations far outweighs the additional cost of feature-card equipment if the capacity of the system is likely to exceed 500 items. A feature-card system specifically applied to inorganic analytical literature and having single sided *Analytical Abstracts* as its foundation has been described. This largely obviates the need for extensive coding of subjects, and makes the index more useful to the analytical chemist in the laboratory.[12]

CONCLUSION

Information retrieval techniques have many applications in the analytical laboratory. It is vital to determine the probable scope required before deciding on the optimum system. Factors to be considered include the type of information available, the information to be retrieved, reasons for requiring the information and the method of seeking it. If a collection of spectra is not to be used to determine the spectrum of compound X, it is unnecessary to include a formula index. When the probable size and nature of material to be included in an index have been determined, the various mechanical means of achieving an efficient form can be considered.

Small systems of transient interest and those to which reference will be made infrequently and from a particular aspect are probably most easily derived by using edge-punched cards. Feature cards are adaptable and most efficient when the population of the system is measured in thousands. The upper capacity limit of a manual system depends again on use.

Anyone considering setting up a system of any size for information retrieval will find study of two books helpful.[21,22] For those wishing to keep abreast of this rapidly growing subject there is the *Journal of Chemical Documentation* issued by the Division of Chemical Literature of the American Chemical Society. A comprehensive review of the state of the subject in the United States has been published,[23] and, in the general field of information retrieval, *ASLIB Proceedings* and the series issued by the National Science Foundation "Current Research and Development in Scientific Documentation" are of interest.

I thank Overseas Geological Surveys for permission to publish this paper.

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Analytical Methods Committee

REPORT PREPARED BY THE ADDITIVES IN ANIMAL FEEDING STUFFS SUB-COMMITTEE

THE Analytical Methods Committee has received the following report from its Additives in Animal Feeding Stuffs Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

In 1958 The Society for Analytical Chemistry received a request from the Scientific Sub-Committee of the Standing Advisory Committee, Fertiliser and Feeding Stuffs Act, 1926, to the Ministry of Agriculture, Fisheries and Food for advice on the availability of suitable methods of analysis for the various types of additives commonly included in animal and poultry feeding stuffs.

The matter was referred to the Analytical Methods Committee of the Society, which appointed the Additives in Animal Feeding Stuffs Sub-Committee to study this problem. The additives involved fall into six principal groups—antibiotics, synthetic hormones, minerals, prophylactics, and water-soluble and oil-soluble vitamins. On the basis of this classification, six panels were set up by the Sub-Committee to investigate suitable methods of analysis for the selected groups of additives.

The growing practice of adding to animal rations not only vitamins and nutritional supplements, but also drugs and medicaments for the stimulation of growth or control of disease has set new problems in analysis and manufacturing control. A particular feature of this development has been the need to bring together two hitherto unrelated fields of analytical activity—that of the pharmaceutical industry on the one hand and of the animal feeding stuffs industry on the other.

Not only has the supplemented feed (as fed to the animal) been considered, but also the higher-potency preparations supplied as diet supplements (pre-mixes), either for intimate admixture by the feeding-stuffs manufacturers or for individual administration by the user. Although analytical methods exist (published or unpublished) for most, if not all, of the substances *per se* used as additives, in few instances has it been established that such methods could be applied to the determination of these additives when present in feeds. To a lesser extent, this also applies to the higher-potency supplements. Broadly speaking, there are three factors to be taken into account when applying an analytical method to feeding stuffs; these are—

(*i*) the sensitivity of the method (because the additive in question may be present at low concentration);

(*ii*) the interference from the ingredients of the basic feed;

(*iii*) the interference from other additives (in some instances, the identity of these may not be known to the analyst).

The representative sampling of feeding stuffs is of considerable importance in the analysis of the products because of the low concentrations of additives usually encountered. This aspect, however, does not strictly come within the purview of the Sub-Committee's work, and so, except in Part 5 of this Report, which deals with the determination of oil-soluble vitamins, no recommendations have been made. In order to assess the precision of any proposed method, each Panel prepared special samples, in addition to using marketed compound feeding stuffs, for collaborative investigations, because experience has shown that extreme care is required in the admixture of additives, and that the degree of fineness of milling can be critical in ensuring the production of a uniform mixed sample for analytical purposes.

This report is divided into six parts, each part dealing with the work of one of the Panels, together with their recommended methods for determining the additives allocated to the particular panel.

PART 1. REPORT OF THE ANTIBIOTICS PANEL

The Determination of Penicillin, Chlortetracycline and Oxytetracycline in Diet Supplements and Compound Feeding Stuffs

INTRODUCTION

THE Antibiotics Panel was set up under the chairmanship of Mr. S. A. Price, and its membership was: Mr. A. J. Cavell, Mrs. J. Gammon, Mr. O. Hughes, Mr. W. P. Jones, Mr. G. Sykes (deputy Miss F. N. Mulholland) and Mr. S. Varsanyi, with Miss A. M. Parry as Secretary; Mr. A. H. Sexton, Mr. J. S. Simpson and Mr. J. H. Taylor also served on the Panel. The Panel was appointed to consider methods for determining antibiotics.

Only three antibiotics—penicillin, chlortetracycline and oxytetracycline—are permitted by law to be added to feeding stuffs in Great Britain. For this purpose they are available as diet supplements, with potencies ranging from about 1 to about 250 g of antibiotic per lb, for incorporation in the feeding stuff at the rate of a few pounds per ton, and as other much less concentrated preparations for incoporation at the rate of several hundredweights per ton. Procedures for determining the three antibiotics in both high-potency and low-potency diet supplements, as well as in compound feeding stuffs, have been devised.

EXPERIMENTAL AND RESULTS

HIGH-POTENCY DIET SUPPLEMENTS—

In investigations of the extraction and assay of supplements the Panel was guided by information from the manufacturers of the products. Early collaborative tests confirmed that chemical as well as microbiological methods were applicable to these relatively high-potency products. The chemical methods recommended by the Panel for each of the three anitbiotics in their respective supplements are described in Appendixes I, II and III, and the results obtained by the collaborating laboratories in a final collaborative test with these methods are summarised in Tables I, II and III. Only one figure for each laboratory is included for a given antibiotic; in some instances this was the result of one determination and in others two or more.

TABLE I

RESULTS OF FINAL COLLABORATIVE TEST OF THE DETERMINATION OF PENICILLIN IN A DIET SUPPLEMENT BY THE RECOMMENDED CHEMICAL METHOD

Sample contained approximately 1 g of procaine penicillin per lb.

Laboratory	Procaine penicillin, g per lb
A	1·15
B	1·03
C	1·02
D	—
E	1·04
F	1·14
G	0·92
Mean	1·05
Standard deviation	0·085
Coefficient of variation	8·1%

From the inter-laboratory variances of these results it was considered that the methods for the assay of high-potency diet supplements are satisfactory, and that, if several laboratories examine samples of this type containing penicillin or chlortetracycline in concentrations of about 1 or 3·6 g per lb., 19 out of 20 of the results might be expected to fall within ±20 per cent. of the means; with supplements containing about 5 g of oxytetracycline per lb, 19 out of 20 of the results might be expected to fall within ±10 per cent. of the mean.

LOW-POTENCY DIET SUPPLEMENTS—

The methods recommended below for compound feeding stuffs are generally suitable for these supplements.

COMPOUND FEEDING STUFFS—

The problems associated with the assays of compound feeding stuffs are much more complex. First, the concentrations of the antibiotics (1 to 20 g per ton) are so low that chemical methods are inapplicable and it is necessary to use microbiological procedures. Second, the composition of feeding stuffs is so variable and the range of additives used is so extensive that a method found satisfactory for one feeding stuff will not necessarily be satisfactory for another.

TABLE II

RESULTS OF FINAL COLLABORATIVE TEST OF THE DETERMINATION OF CHLORTETRACYCLINE IN A DIET SUPPLEMENT BY THE RECOMMENDED CHEMICAL METHOD

Sample contained approximately 3·5 g of chlortetracycline hydrochloride per lb.

Laboratory	Chlortetracycline hydrochloride, g per lb
A	3·37
B	3·30
C	3·10
D	—
E	3·53
F	3·52
G	2·75
Mean	3·26
Standard deviation	0·296
Coefficient of variation	9·1%

TABLE III

RESULTS OF FINAL COLLABORATIVE TEST OF THE DETERMINATION OF OXYTETRACYCLINE IN A DIET SUPPLEMENT BY THE RECOMMENDED CHEMICAL METHOD

Sample contained approximately 5 g of oxytetracycline per lb.

Laboratory	Oxytetracycline (free base), g per lb
A	4·76
B	4·81
C	4·73
D	—
E	4·83
F	4·83
G	4·73
Mean	4·78
Standard deviation	0·176
Coefficient of variation	3·7%

At the beginning of the work the Panel agreed on the use of plate, rather than tube, methods for the microbiological assays, and the use of large plates rather than Petri dishes was preferred. It was also decided that the extraction methods to be used should be those that had been found satisfactory for the respective supplements by the antibiotic manufacturers themselves. For the early investigations, these extraction methods and the organisms to be used were defined, but the details of the microbiological techniques were left to the choice of the individual analysts.

The results of these trials were encouraging with penicillin and oxytetracycline, but with chlortetracycline it was apparent that the procedure had to be investigated more fully if accurate and precise results were to be obtained. Experiments were therefore undertaken to find the optimum conditions for plate assays, particularly as applied to chlortetracycline.

Among the factors examined were the constituents of the assay medium and its depth, the cavity size, the strain of test organism and whether or not the inoculum should be vegetative or a spore suspension, and the incubation temperature. Inocula and media were exchanged, and a detailed examination was made of the size and clarity of the inhibition zones in relation to the results obtained. It was evident from this work that the methods for all three antibiotics should be specified in considerable detail.

With some samples it is desirable that an unsupplemented sample of the feeding stuff under test should be available as a blank sample; it can then be extracted by exactly the same procedure as is applied to the test sample, and the extract used as a diluent for the standard, *i.e.*, to give a "modified standard." As an unsupplemented sample is rarely available in practice, the Panel also investigated methods for destroying or removing the antibiotic from the sample or the sample extract: the procedures found satisfactory for this purpose are included in the methods recommended for the assay of compound feeding stuffs described in Appendixes IV, V and VI. The methods drafted by the Panel specify in unambiguous detail the procedure to be followed in the extraction stage, but at the same time are sufficiently flexible to be carried out in any laboratory equipped for, and experienced in, this type of work. Throughout the collaborative studies, the Panel has been impressed by the importance of experience, not only in microbiological assays in general, but in the assay of each particular antibiotic. It is assumed also that anyone undertaking such assays is familiar with the statistical design and calculations involved. The results obtained by the collaborating laboratories in a final collaborative test with these methods are summarised in Tables IV, V and VI.

TABLE IV

RESULTS OF ASSAYS OF PENICILLIN IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

Key to Standards—Standard 1—Unmodified.
Standard 2—Modified with unsupplemented poultry meal extract.
Standard 3—Modified with supplemented poultry meal extract treated to remove the antibiotic.
Standard 4—Modified with unsupplemented pig meal extract.
Standard 5—Modified with supplemented pig meal extract treated to remove the antibiotic.

	Penicillin in poultry meal			Penicillin in pig meal		
Laboratory	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	5·55	5·50	5·21	5·05	5·28	4·90
B	6·10	6·10	5·50	5·57	5·90	5·60
C	5 40	5·29	5·70	5·10	5·30	5·50
D	5·12	5·34	—	5·05	5·58	—
E	5·19	5·59	5·15	5·07	5·12	5·17
F	5·63	5·51	5·49	5·36	5·52	5·23
Mean	5·50	5·55	5·41	5·20	5·45	5·28
Standard deviation ..	0·35	0·29	0·23	0·22	0·28	0·28
Coefficient of variation ..	6·46%	5·23%	4·25%	4·17%	5·14%	5·31%

At first sight it would appear from the Tables that the results outlined with unmodified standards do not differ materially from those obtained with modified standards. In some laboratories however, the simpler procedure, in which unmodified standards were used, occasionally resulted in invalid assays, because the dose - response curves were non-parallel. Provided that there is no such deviation from parallelism between the slopes, the simpler procedure, in which the standard is diluted with buffer solution rather than with unsupplemented or treated feed extract, may be used.

Some collaborating laboratories found evidence of non-parallelism of the response curves when unmodified standards were used; the means and standard deviations of results so obtained have not, therefore, been included in the Tables.

It must be emphasised that in using these methods it is assumed that the identity of the antibiotic is known and that only one is present. The determination of two or more antibiotics present together in a feeding stuff is likely to present special problems to the analyst, and the presence of certain prophylactics and other drugs may complicate otherwise satisfactory microbiological methods. These are problems that could well occupy attention in the future, but their complete solution will probably depend on more detailed fundamental work as well as on collaborative investigation.

From the inter-laboratory variances shown in Tables IV, V and VI it may be stated that, if several laboratories experienced in microbiological assays of this type all assay the same samples of poultry or pig meals containing 5 g of penicillin or 10 g of chlortetracycline or oxytetracycline per ton, 19 out of 20 results may be expected to be within about 10 per cent. of the mean for penicillin, 20 per cent. of the mean for chlortetracycline and 25 per cent. of the mean for oxytetracycline.

TABLE V

RESULTS OF ASSAYS OF CHLORTETRACYCLINE IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

For Key to Standards—See Table IV

Laboratory	Chlortetracycline in poultry meal			Chlortetracycline in pig meal		
	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	8·51	9·95	9·90	7·41	9·77	8·90
B	10·00	—	9·20	9·30	—	8·60
C	8·34	8·99	8·91	9·27	9·35	9·35
D	9·84	10·01	9·39	9·27	9·64	8·74
E	7·97	9·05	8·97	10·02	9·75	10·03
F	7·67	8·91	7·14	8·47	8·90	8·33
Mean	8·72	9·38	8·92	8·96	9·48	8·99
Standard deviation ..	0·97	0·55	0·86	0·90	0·37	0·61
Coefficient of variation..	11·2%	5·87%	9·64%	10·06%	3·91%	6·79%

TABLE VI

RESULTS OF ASSAYS OF OXYTETRACYCLINE IN COMPOUND FEEDING STUFFS MEASURED AGAINST VARIOUS STANDARDS

For Key to Standards—See Table IV

Laboratory	Oxytetracycline in poultry meal			Oxytetracycline in pig meal		
	Standard 1, g per ton	Standard 2, g per ton	Standard 3, g per ton	Standard 1, g per ton	Standard 4, g per ton	Standard 5, g per ton
A	7·89	8·98	8·71	7·85	8·76	8·78
B	9·15	11·75	11·45	9·10	10·85	11·10
C	9·53	9·37	9·42	9·32	9·31	9·10
D	7·66	9·29	8·69	8·39	10·0	9·17
E	9·21	9·66	9·80	8·80	9·18	9·72
F	11·05	11·40	10·70	9·73	9·87	9·80
Mean	9·08	10·07	9·79	8·86	9·66	9·61
Standard deviation ..	1·23	1·18	1·10	0·67	0·74	0·83
Coefficient of variation..	13·5%	11·76%	11·28%	7·61%	7·66%	8·6%

ACKNOWLEDGMENTS

The Panel is grateful to the organisations in whose laboratories the work was carried out, and in particular to Messrs. Boots Pure Drug Co. Ltd., Cyanamid of Great Britain Ltd., Distillers Company Ltd., Glaxo Laboratories Ltd., Pfizer Ltd. and Vitamins Ltd. for generously supplying materials for the collaborative tests.

Appendix I

CHEMICAL DETERMINATION OF PENICILLIN IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The supplement is extracted with water, and the procaine penicillin in the extract is determined by the iodimetric method of the "British Pharmacopoeia 1958"[1] for total penicillins in benzylpenicillin (see Note 1).

APPLICABILITY AND RANGE—

The method is applicable to diet supplements containing not less than 1 g of penicillin per lb.

REAGENTS—

Water—Use distilled or de-ionised water.

The other reagents required are described in the "British Pharmacopoeia 1958."[1]

PROCEDURE

EXTRACTION OF SAMPLE—

(*a*) *For supplements containing* 1 *to* 5 *g of penicillin per lb*—Weigh accurately about 25 g of sample, and transfer quantitatively to a small glass mortar. Mix with a few millilitres of water, and grind to form a smooth paste. Transfer the mixture quantitatively to a tared 250-ml conical flask, rinsing in with water, and add sufficient water to the mixture to give a total weight of 175 g. Mix thoroughly, and set aside for 5 minutes, with occasional shaking. Filter the supernatant liquid through a fluted 24-cm Whatman No. 42 filter-paper, discard the first 20 ml of filtrate, and then collect 60 ml. Determine the procaine penicillin in the filtrate by the method described below (see Note 2).

(*b*) *For supplements containing* 16 *g of penicillin per lb*—Extract the sample as described above under (*a*), but use about 12 g of sample and make the weight of the mixture up to 162 g with water.

(*c*) *For supplements containing* 224 *g of penicillin per lb*—Weigh accurately about 2·5 g of sample, and transfer quantitatively to a glass mortar. Mix with a few millilitres of water, and grind to form a smooth paste. Transfer the mixture quantitatively to a 500-ml calibrated flask, dilute to about 400 ml with water, and set aside for 5 minutes, with occasional shaking. Dilute the mixture to the mark, mix well, immediately filter through a fluted 24-cm Whatman No. 42 filter-paper, discard the first 20 ml of filtrate, and then collect 60 ml. Determine the procaine penicillin in the filtrate by the method described below (see Note 2).

DETERMINATION OF PROCAINE PENICILLIN—

Determine the procaine penicillin in a 10-ml portion of the filtrate from the extraction of the sample by the iodimetric method for total penicillins exactly as described in the "British Pharmacopoeia 1958" under "Benzylpenicillin," p. 89 (see Note 3).

RESULTS—

Express the results as grams of procaine penicillin per lb of supplement.

NOTES

1. Since this report was prepared the "British Pharmacopoeia 1963"[2] has been published, and in this new pharmacopoeia a method is included for the determination of total penicillins in procaine penicillin. The B.P., 1963, method is similar to that recommended by the Panel, except that the procaine is first removed from solution by precipitation with sodium silicotungstate; as the procaine has been removed, it is unnecessary to apply the correction factor of 1·04 (see Note 3).

2. The analysis should be completed with the minimum of delay, because the aqueous penicillin solution slowly decomposes.

3. It is necessary, in order to determine the factor applicable in each laboratory, to standardise the volumetric reagents by using a standard preparation of benzylpenicillin sodium and to convert the factor obtained to a figure applicable to procaine penicillin by multiplying by the appropriate molecular-weight ratio; the molecular weight of benzylpenicillin sodium is 356·4 and that for procaine penicillin (monohydrate) is 588·7, so that the ratio of molecular weights is 1 to 1·652. It is generally recognised that when the iodimetric method is applied to procaine penicillin it gives results that are 4 per cent. low; it is recommended, therefore, that the factor be multiplied by 1·04 to correct for this.

Appendix II

CHEMICAL DETERMINATION OF CHLORTETRACYCLINE IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The method is based on that of Chiccarelli, Woolford and Trombitas.[3]

REAGENTS—

Water—Use distilled or de-ionised water.

Hydrochloric acid, 5 N.

Dilute sodium hydroxide solution—Dilute 8 ml of 5 N sodium hydroxide to 100 ml with water.

Sodium metabisulphite solution—A 10 per cent. w/v solution in water. The solution must be freshly prepared.

Phosphate buffer solution (*pH* 7·5)—Dissolve 178 g of dipotassium hydrogen orthophosphate, K_2HPO_4, and 22 g of potassium dihydrogen orthophosphate, KH_2PO_4, in 1 litre of water. Filter the solution before use.

Stock standard chlortetracycline solution—Transfer exactly 100 mg of chlortetracycline hydrochloride B.P. to a 100-ml calibrated flask, dilute to the mark at 20° C with water, and mix well. Store the solution in an amber-glass bottle at 5° to 8° C; under these conditions the solution is stable for one week.

Working standard chlortetracycline solution—Dilute 5·0 ml of stock standard chlortetracycline solution to 100 ml at 20° C with water, and mix well;

1 ml ≡ 0·05 mg of chlortetracycline hydrochloride.

Prepare the solution immediately before use.

PROCEDURE

EXTRACTION OF SAMPLE—

Weigh accurately a portion of the sample expected to contain approximately 5 mg of chlortetracycline hydrochloride (see Note 1) into a 100-ml calibrated flask. Add about 70 ml of water and 4 ml of 5 N hydrochloric acid, shake the mixture for 10 minutes, dilute to the mark with water, and mix thoroughly (see Note 2).

Filter a portion of the mixture through Celite filter aid, discarding the first 20 to 30 ml of filtrate, collect the clear filtrate, and determine the chlortetracycline in the filtrate by the method described below.

DETERMINATION OF CHLORTETRACYCLINE—

Transfer by pipette two 10-ml portions of the filtrate from the extraction of the sample into separate 50-ml calibrated flasks; these form the sample and sample blank solutions.

Transfer by pipette two 10-ml portions of the working standard chlortetracycline solution into further separate 50-ml calibrated flasks; these form the standard and standard blank solutions.

To the solutions representing the sample and the standard add, in order, 12 ml of 5 N hydrochloric acid, 15 ml of phosphate buffer solution (pH 7·5), 2 ml of sodium metabisulphite solution and 3 ml of dilute sodium hydroxide solution, and suspend the flasks in a bath of boiling water for exactly 7 minutes, swirling the contents occasionally (see Note 3).

To the solutions representing the sample blank and the standard blank add 15 ml of phosphate buffer solution (pH 7·5), 2 ml of sodium metabisulphite solution and 3 ml of dilute sodium hydroxide solution, and suspend the flask in a bath of boiling water, swirling the contents occasionally (see Note 3). After exactly 5 minutes, add 12 ml of 5 N hydrochloric acid, and heat for an additional 2 minutes.

Immediately after the completion of the heat treatment, cool the four flasks in running water, dilute the contents of each flask to the mark at 20° C with water, and mix well.

If a cloudiness develops in the solutions at this point, spin the sample and sample blan solutions in a centrifuge until clear.

Measure the optical densities, at 445 mμ, of the solutions representing the standard an the sample against their respective blank solutions with a suitable spectrophotometer.

CALCULATION—

The amount of chlortetracycline hydrochloride in the sample is given by the expression—

Chlortetracycline hydrochloride content =

$$\frac{(A_{445}\ \text{sample}) \times 0{\cdot}01 \times (\text{dilution of sample}) \times 453{\cdot}6}{(A_{445}\ \text{standard}) \times (\text{weight of sample}) \times 1000}\ \text{g per lb},$$

where A_{445} = optical density at 445 mμ and

0·01 = final concentration of the standard in mg per ml.

RESULTS—

Express the results as grams of chlortetracycline hydrochloride per lb of supplement.

NOTES

1. The sample taken must not weigh less than 100 mg. If high-potency material is to be assayed and a weight of 100 mg contains more than 5 mg of chlortetracycline hydrochloride, take a sample weighing 100 mg or more, and make appropriate dilutions to give a concentration of 0·05 mg per ml. Increase the amount of 5 N hydrochloric acid used in direct proportion to the dilution.
2. The concentration of this solution is approximately 0·05 mg of chlortetracycline hydrochloride per ml.
3. It is essential that the water is boiling throughout the entire heating period.

Appendix III

CHEMICAL DETERMINATION OF OXYTETRACYCLINE IN DIET SUPPLEMENTS

PRINCIPLE OF METHOD—

The method is based on the measurement of the colour produced by reaction of oxytetracycline with ferric chloride in the presence of 0·01 N hydrochloric acid.

APPLICABILITY—

Ingredients such as phosphates, fluoride, thiocyanates, etc., that combine with ferric iron, interfere with the method. These interfering substances must therefore be absent or be removed before the analysis is undertaken.

RANGE—

For supplements containing not less than 5 g of oxytetracycline per lb.

REAGENTS—

Water—Use distilled or de-ionised water.

Dilute acetic acid—A 10 per cent. v/v solution of glacial acetic acid in water.

Concentrated hydrochloric acid, sp.gr. 1·16 *to* 1·18.

Hydrochloric acid, 0·01 N—Adjust to pH 2·0 if necessary.

Ferric chloride solution—A 0·05 per cent. w/v solution of ferric chloride, $FeCl_3.6H_2O$, in 0·01 N hydrochloric acid.

Standard oxytetracycline solution—Dissolve sufficient oxytetracycline hydrochloride in 0·01 N hydrochloric acid, and dilute with the same acid to give a solution containing 400 μg of oxytetracycline per ml.

PROCEDURE

EXTRACTION OF SAMPLE—

(*a*) *For supplements containing* 5 g *of oxytetracycline per lb*—Weigh accurately about 3 g of the sample into a 250-ml beaker, add 50 ml of acetic acid, and set the mixture aside for 20 minutes, stirring gently (see Note 1). Filter the solution quantitatively through a Buchner funnel, more than once if necessary, until the filtrate is absolutely clear. Wash the filter-paper with 10 ml of water, combine the filtrate and washings, and adjust the clear mixture to pH 2·0 ± 0·05 (measured with a pH meter) with a few drops of concentrated hydrochloric acid. Transfer the solution quantitatively to a 100-ml calibrated flask, rinsing in with 0·01 N hydrochloric acid, and dilute to the mark at 20° C with the same acid (see Note 2). Determine the oxytetracycline hydrochloride in the solution by the method described below.

(*b*) *For supplements containing* 10 g *of oxytetracycline per lb*—Extract the sample as described under (*a*), but use about 2·5 g of sample.

(*c*) *For supplements containing* 25 g *of oxytetracycline per lb*—Extract the sample as described under (*a*), but use about 1 g of sample.

DETERMINATION OF OXYTETRACYCLINE—

Transfer a 5-ml portion of the solution from the extraction of sample to each of two clean test-tubes. Add to one tube 15 ml of 0·01 N hydrochloric acid and to the other tube 5 ml of 0·01 N hydrochloric acid and 10 ml of ferric chloride solution, mix, and set aside at 20° to 25° C for 20 minutes. Measure the optical density of each solution in turn at 490 mμ., in a 1-cm cell with a suitable spectrophotometer.

Prepare, in 0·01 N hydrochloric acid, dilutions of standard oxytetracycline solution covering the range 0 to 400 μg of oxytetracycline per ml, and treat 5-ml portions of each dilution as described above for the solution from the extraction of the sample. Construct a graph relating the optical densities to the number of micrograms of oxytetracycline in each dilution.

By reference to this calibration graph, determine the amount of oxytetracycline in the solution from the extraction of the sample, and hence calculate the amount of oxytetracycline in the sample.

RESULTS—

Express the result as grams of oxytetracycline (free base) pei lb of supplement.

NOTES

1. A magnetic stirrer is suitable.
2. If the material being assayed contains 5 g of oxytetracycline per lb, the concentration in this solution is approximately 300 μg per ml.

Appendix IV

MICROBIOLOGICAL ASSAY OF PENICILLIN IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium—

Peptone	5·0 g
Lab. Lemco	3·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the peptone and the Lab. Lemco in the water, adjust the solution to pH 7·0, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes.

Acetone - phosphate buffer solution (*pH* 7·5)—Dissolve 9·78 g of disodium hydroge orthophosphate, Na_2HPO_4, and 1·85 g of potassium dihydrogen orthophosphate, KH_2PO_4 in 200 ml of hot distilled water, cool, add 250 ml of acetone, and dilute to 1 litre with distille water.

Phosphate buffer solution (*pH* 7·0)—Dissolve 5·0 g of dipotassium hydrogen orthophosphate, K_2HPO_4, and 3·9 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water, and dilute to 5 litres with distilled water. The solution may be stored; if so, it should be sterilised.

ORGANISM—

The organism used in the assay is *Bacillus subtilis* (NCIB 8236), or any other suitabl strain, *e.g.*, ATCC 6633.

Maintenace of culture—Maintain cultures of the organism on slopes of either of the two sterile media listed below—

Peptone	6·0 g
Casein hydrolysate (enzymic)	4·0 g
Marmite	3·0 g
Lab. Lemco	1·5 g
Dextrose	1·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

Adjust to pH 6·5 to 6·6, and sterilise by heating at 115° C for 10 minutes in 10-ml bottles.

or

Difco Penassay Seed Agar

Sterilise by heating at 115° C for 10 minutes in 10-ml bottles.

Preparation of spore suspension—Transfer 200 ml of one of the media to a Roux bottle, and sterilise by heating at 115° C for 10 minutes. Cool, inoculate with the culture, and incubate at approximately 30° C for 7 to 8 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, spin the suspension in a centrifuge, re-suspend the deposit in sterile glass-distilled water, and dilute to match approximately opacity 4 on Brown's opacity tubes. Fill 1-oz screw-capped bottles with the suspension, pasteurise at 70° to 75° C for 30 minutes, and store in a refrigerator.

PROCEDURE

PREPARATION OF PLATES—

Melt the contents of a bottle of assay medium by steaming for approximately 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. subtilis*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2)—mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of benzylpenicillin sodium of known potency in phosphate buffer solution (pH 7·0), and dilute to 500 ml with the buffer solution (see Note 4).

Take a portion of the solution containing as much antibiotic activity as is estimated to be present in the test sample (*see Note* 5), *dilute to* 100 *ml with acetone - phosphate buffer solution* (*pH* 7·5), *and add* 20 g *of the blank* (*unsupplemented*) *feed* (*see Note* 6). *Shake for* 1 *hour, and then spin in a centrifuge, or filter.* Take a portion of the solution, dilute, if necessary, with acetone - phosphate buffer solution (pH 7·5) to give a solution containing 1·0 i.u. of penicillin per ml, and from this make a further dilution in acetone - phosphate buffer solution (pH 7·5) to give a solution containing 0·2 i.u. of penicillin per ml.

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add 250 ml of acetone - phosphate buffer solution (pH 7·5), and shake vigorously. Spin in a centrifuge, or filter, and dilute the solution with acetone - phosphate buffer solution (pH 7·5) in accordance with the expected potency to the levels required in the assay, namely, approximately 1·0 and 0·2 i.u. of penicillin per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of acetone - phosphate buffer solution (pH 7·5), and shake for 1 hour. Spin in a centrifuge, or filter, and dilute the solution with acetone - phosphate buffer solution (pH 7·5) according to the expected potency to the levels required in the assay, namely, approximately 1·0 and 0·2 i.u. of penicillin per ml.

ASSAY—

By means of a statistically satisfactory assay design, such as suggested by Brownlee *et al.*,[4] Lees and Tootill,[5] Price and Boucher[6] or Simpson and Lees,[7] compare the test and standard solutions in a cup-plate assay by using the previously prepared plates.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses, and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$\text{antilog}\left\{\frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 5\right\},$$

where T_2 = the total of the responses to the high dose of test solution,

T_1 = the total of the responses to the low dose of test solution,

S_2 = the total of the responses to the high dose (1·0 i.u. per ml) of standard solution, and

S_1 = the total of the responses to the low dose (0·2 i.u. per ml) of standard solution

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of procaine penicillin per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of the medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by the Panel members ranged from 15 to 28 mm for a dose level of 1·0 i.u. per ml and from 10 to 20 mm for a dose level of 0·2 i.u. per ml. The increase in response for this five-fold increase in dose ranged from 5 to 10 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of diet supplements, the instructions in *italic type* can be omitted. They may also be omitted in the assay of compound feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an "unmodified standard."

5. Take a 0·6-ml portion for a feeding stuff estimated to contain 5 g of procaine penicillin per ton.

6. If unsupplemented feed is not available, a suitable preparation can be made by steaming a portion of the test sample for 1 hour or heating in an autoclave at 120° C for 15 minutes.

Appendix V

MICROBIOLOGICAL ASSAY OF CHLORTETRACYCLINE IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium —

Yeast extract	1·0 g
Ammonium nitrate	5·0 g
Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), B.P. grade	5·0 g
Dextrose	5·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the yeast extract, ammonium nitrate and sodium dihydrogen orthophosphate in the water, adjust the solution to pH 7·5, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Remove from the autoclave, add the dextrose and filter clear through a prepared paper-pulp pad about 0·5 inch thick. Cool, and adjust the solution to pH 7·0. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes. The final pH of the medium is 6·7.

Aqueous acid - acetone mixture—Mix 40 ml of hydrochloric acid, sp.gr. 1·16 to 1·18, with 1300 ml of acetone, and dilute to 2 litres with distilled water.

Sodium hydroxide, N.

Phosphate buffer solution (*pH* 4·5)—Dissolve 13·6 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water, and dilute to 1 litre with distilled water.

Hydrochloric acid, N.

Sodium chloride solution—A 5·85 per cent. w/v solution in distilled water.

ORGANISM—

The organism used in the assay is *Bacillus cereus* NCIB 8849 or 9231 (ATCC 11778).

Maintenance of culture—Maintain cultures of the organism on slopes of "Oxoid" Blood Agar Base ("Oxoid" Nutrient Broth + 1·5 per cent. of agar) that has been sterilised at 115° C for 10 minutes in 10-ml screw-capped bottles.

Preparation of spore suspension—Prepare the following medium—

Protone (Difco)	5·0 g
Manganese sulphate ($MnSO_4.4H_2O$)	0·001 g
Agar	20·0 g
Glass-distilled water	to 1 litre

Transfer 200-ml amounts of the medium to Roux bottles, and sterilise by heating at 120° C for 15 minutes. Cool, inoculate with the culture, and incubate at 30° C for 5 to 7 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, and spin the suspension in a centrifuge; wash the deposit with three successive 150-ml portions of sterile glass-distilled water, spinning the suspension in a centrifuge each time. Re-suspend the growth in sterile glass-distilled water, and dilute to match opacity 1, or less, on Brown's opacity tubes. Store the suspension in a refrigerator.

PROCEDURE

PREPARATION OF PLATES—

Melt the contents of a bottle of assay medium by steaming for approximately 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. cereus*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2), mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of chlortetracycline hydrochloride of known potency in phosphate buffer solution (pH 4·5), and dilute to 500 ml with the buffer solution (see Note 4).

Measure 25 ml of solution A (see below under "Preparation of Test Solutions") into a 50-ml beaker, add 2·5 ml of N *sodium hydroxide, boil gently for 15 minutes, and cool. Add 2·5 ml of* N *hydrochloric acid, transfer the solution quantitatively to a 50-ml calibrated flask, add a portion of the standard chlortetracycline solution containing as much antibiotic activity as is estimated to be present in the test sample, and dilute to the mark with phosphate buffer solution (pH 4·5).* Dilute a portion with phosphate buffer solution (pH 4·5) to give a solution containing 0·25 μg of chlortetracycline per ml, and from this make a further dilution in phosphate buffer solution (pH 4·5) to give a solution containing 0·05 μg of chlortetracycline per ml.

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add sufficient aqueous acid - acetone mixture to give a volume of 250 ml, shake for 1 hour, and filter. Dilute the filtrate with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·25 and 0·05 μg of chlortetracycline per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of aqueous acid - acetone solution, shake for 1 hour, and spin in a centrifuge. Titrate a 5-ml portion of the supernatant liquid with N sodium hydroxide, with methyl orange solution as indicator. Transfer a 25-ml portion of the supernatant liquid to a 100-ml calibrated flask, add a volume of N sodium hydroxide equivalent to five times the volume required in the titration, and dilute to the mark with phosphate buffer solution (pH 4·5). (This is Solution A.)

To 10 ml of Solution A, add 1 ml of sodium chloride solution, and dilute with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·25 and 0·05 μg of chlortetracycline per ml, (see Note 5).

ASSAY—

By means of a statistically satisfactory assay design, such as suggested by Brownlee *et al.*,[4] Lees and Tootill,[5] Price and Boucher[6] or Simpson and Lees,[7] compare the test and standard solutions in a cup-plate assay by using the previously prepared plates.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses, and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$P = 0{\cdot}25 \text{ antilog} \left\{ \frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 5 \right\},$$

where T_2 = the total of the responses to the high dose of test solution,

T_1 = the total of the responses to the low dose of test solution,

S_2 = the total of the responses to the high dose (0·25 μg per ml) of standard solution and

S_1 = the total of the responses to the low dose (0·05 μg per ml) of standard solution.

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of chlortetracycline hydrochloride per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by the Panel members ranged from 14 to 30 mm for dose levels of 0·25 μg per ml and from 12 to 25 mm for dose levels of 0·05 μg per ml. The increase in response for this five-fold increase in dose ranged from 2·2 to 9 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of diet supplements the instructions in *italic type* can be omitted. They may also be omitted in the assay of compound feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an unmodified standard.

5. If, for example, the sample contains 10 g per ton, or 9·84 μg per g, dilute to 20 ml.

Appendix VI

MICROBIOLOGICAL ASSAY OF OXYTETRACYCLINE IN DIET SUPPLEMENTS AND COMPOUND FEEDING STUFFS

Normal bacteriological procedures and precautions must be adopted in preparation of cultures, sterilisation of media and glassware, etc.

REAGENTS—

Assay medium—

Yeast extract	1·0 g
Ammonium nitrate	5·0 g
Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), B.P. grade	5·0 g
Dextrose	5·0 g
Agar	15·0 g
Glass-distilled water	to 1 litre

The recommended procedure for preparing the medium is to dissolve the yeast extract, ammonium nitrate and sodium dihydrogen orthophosphate in the water, adjust the solution to pH 7·5, stir in the agar, and transfer the mixture to an autoclave. Steam for 30 minutes, and then heat at 115° C for 20 minutes. Remove from the autoclave, add the dextrose, and filter clear through a prepared paper-pulp pad about 0·5 inch thick. Cool, and adjust the solution to pH 7·0. Transfer 200- to 250-ml amounts (see Note 1) to 12-oz screw-capped bottles, and sterilise at 115° C for 20 minutes. The final pH of the medium is 6·7.

Acid methanol solution, 2 per cent. v/v—Dilute 20 ml of hydrochloric acid, sp.gr. 1·16 to 1·18, to 1 litre with methanol.

Sodium hydroxide, N.

Hydrochloric acid, N.

Phosphate buffer solution (pH 4·5)—Dissolve 13·6 g of potassium dihydrogen orthophosphate, KH_2PO_4, in distilled water and dilute to 1 litre with distilled water.

Sil Flo—Obtainable from Haller and Phillips Ltd., 68–70 Goswell Road, London, E.C.1.

Sodium carbonate—$Na_2CO_3.10H_2O$. B.P. quality.

ORGANISM—

The organism used in the assay is *Bacillus cereus* NCIB 8849 or 9231 (ATCC 11778).

Maintenance of culture—Maintain cultures of the organism on slopes of "Oxoid" Blood Agar Base ("Oxoid" Nutrient Broth + 1·5 per cent. of agar) that has been sterilised at 115° C for 10 minutes in 10-ml screw-capped bottles.

Preparation of spore suspension—Prepare the following medium—

Protone (Difco)	5·0 g
Manganese sulphate ($MnSO_4.4H_2O$)	0·001 g
Agar	20·0 g
Glass-distilled water	to 1 litre

Transfer 200-ml amounts of the medium to Roux bottles, and sterilise by heating at 120° C for 15 minutes. Cool, inoculate with the culture, and incubate at 30° C for 5 to 7 days. Wash the growth from the surface of the medium with 150 ml of sterile glass-distilled water, and spin the suspension in a centrifuge; wash the deposit with three successive 150-ml portions of sterile glass-distilled water, spinning the suspension in a centrifuge each time. Re-suspend the deposit in sterile glass-distilled water, and dilute to match opacity 1, or less, on Brown's opacity tubes. Store the suspension in a refrigerator.

PROCEDURE

PREPARATION OF THE PLATES—

Melt the contents of a bottle of assay medium by steaming for about 1 hour, and cool to about 50° C in a water bath at this temperature.

Inoculate the melted medium with the spore suspension of *B. cereus*—dilute the suspension, if necessary, and use an inoculum density found by previous experiment to give zone diameters of suitable size (see Note 2)—mix well, and pour on to a 12-inch × 12-inch sterilised plate (see Note 3). The plate must be supported on a level surface while the medium is being poured. Leave the plate at room temperature with the lid raised until the medium has set, and store in a refrigerator for at least 1 hour.

Cut 64 cups in the solidified medium with the aid of a template; use a No. 5 cork-borer (8 mm in diameter) or nearest available size.

PREPARATION OF STANDARD SOLUTIONS—

Dissolve 50 mg of oxytetracycline hydrochloride of known potency in phosphate buffer solution (pH 4·5), dilute to 500 ml with the buffer solution and prepare modified standards by either method (*i*) or (*ii*) described below (see Note 4).

(*i*) *Standard solution modified with inactivated supplemented feed*—Prepare an adsorption column, 9 to 10 cm in length, composed of a mixture of equal parts of sodium carbonate and Sil Flo, in a chromatography tube, 3 cm in diameter. Pack the column dry, and tamp down before use.

Transfer approximately 50 ml of Solution A (see below under "Preparation of Test Solutions") to the top of the dry adsorption column, allow the solution to pass through the column, and finally apply gentle suction. Pass the solution through the column once only. Adjust the pH of the eluate to pH 4·5 with N hydrochloric acid, and transfer 20 ml of the solution quantitatively to a 50-ml calibrated flask. Add a portion of the standard oxytetracycline solution, prepared as described above, containing as much antibiotic activity as is estimated to be present in 4·0 g of the test sample, and dilute to the mark with phosphate buffer solution (pH 4·5). Dilute a portion with phosphate buffer solution (pH 4·5) to give a solution containing 0·8 μg of oxytetracycline per ml, and from this make a further dilution in phosphate buffer solution (pH 4·5) to give a solution containing 0·2 μg of oxytetracycline per ml.

(*ii*) *Standard solution modified with unsupplemented feed*—Weigh a 20-g sample of the unsupplemented feeding stuff, add 100 ml of acid methanol, shake for 1 hour, and spin in a centrifuge. Adjust the supernatant liquid to pH 4·5 with N sodium hydroxide, and transfer 20 ml of the solution quantitatively to a 50-ml calibrated flask. Add a suitable portion of the standard oxytetracycline solution, and dilute as described above under (*i*).

PREPARATION OF TEST SOLUTIONS—

Supplements—Weigh a 1-g sample, add sufficient acid methanol to give a volume of 250 ml, shake for 1 hour, and filter. Dilute the filtrate with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·8 and 0·2 μg of oxytetracycline per ml.

Feeding stuffs—Weigh a 20-g sample, add 100 ml of acid methanol, shake for 1 hour, and spin in a centrifuge. (This is Solution A.)

Transfer 10 ml of Solution A to a 25-ml calibrated flask, adjust the pH to 4·5 with N sodium hydroxide, and dilute to the mark with phosphate buffer solution (pH 4·5). If necessary, dilute portions with phosphate buffer solution (pH 4·5) according to the expected potency to the levels required in the assay, namely, approximately 0·8 and 0·2 μg of oxytetracycline per ml.

ASSAY—

Compare the test and standard solutions in a cup-plate assay; use the previously prepared plates and a statistically satisfactory assay design.

Plate out the solutions according to the chosen assay design; deliver a uniform volume of 0·05 to 0·1 ml into each cup of the prepared plates. Set aside at room temperature for 1 hour to allow pre-diffusion, and then place in an incubator at approximately 30° C.

After incubation for 18 to 20 hours, measure the diameters of the inhibition zones with finely pointed vernier callipers or a projection device.

Check the parallelism of the test and standard responses and, if satisfactory, calculate the potency, P, of the test solution from the equation—

$$P = 0{\cdot}8 \text{ antilog}\left\{ \frac{(T_2 + T_1) - (S_2 + S_1)}{(S_2 - S_1) + (T_2 - T_1)} \times \log 4 \right\},$$

where T_2 = the total of the responses to the high dose of test solution,
T_1 = the total of the responses to the low dose of test solution,
S_2 = the total of the responses to the high dose (0·8 μg per ml) of standard solution and
S_1 = the total of the responses to the low dose (0·2 μg per ml) of standard solution.

Calculate the potency of the sample from the dilution employed.

RESULTS—

Express the results as grams of oxytetracycline (base) per pound of diet supplement or per ton of compound feeding stuff.

NOTES

1. The volume of medium should be such that when poured into a 12-inch × 12-inch plate the depth of medium is 0·1 inch.

2. Zone diameters reported by Panel members ranged from 16 to 26 mm for dose levels of 0·8 μg per ml and from 11 to 18 mm for dose levels of 0·2 μg per ml. The increase in response for this four-fold increase in dose ranged from 3·2 to 10 mm.

3. Sterilisation may be achieved by heating in an autoclave or by swabbing with acid alcohol.

4. For the assay of supplements a standard diluted further with the buffer solution to give solutions containing 0·8 and 0·2 μg of oxytetracycline per ml may be used instead of one modified with a feeding stuff as described below. Likewise, with feeding stuffs if, by previous experiment, it has been found that the test response is parallel to that of an unmodified standard, a modified standard need not be used.

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The Determination of Small Amounts of Iron and Chromium in Sapphire and Ruby for Maser Applications

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It has been required to know the amount and distribution of paramagnetic ions in synthetic sapphire and ruby crystals used for maser devices. The extremely hard and chemically inert nature of the sample materials creates special analytical problems.

Methods, both chemical and spectrographic, have been developed for determining 0·002 to 0·2 per cent. of iron and of 0·01 to 0·5 per cent. of chromium. The methods are applicable to small samples ($\not>$ 20 mg) so that material from different regions of a crystal can be analysed to assess homogeneity.

In the spectrographic method, the crushed sample is mixed with graphite powder containing cobalt oxide as internal standard, and a d.c. arc total-combustion technique is used to determine chromium and iron. The chemical methods involve spectrophotometric determination of chromium with diphenylcarbazide and of iron with bathophenanthroline, both applied after decomposition of the crushed sample with a sodium carbonate - sodium tetra-borate flux; for determining iron at low levels rigorous purification of reagents is essential to achieve suitably low blank values.

The chemical methods have higher precision, but the spectrographic method is more rapid and is preferred for routine work. Results by the various methods are presented.

SAPPHIRES and rubies have long been prized as gemstones. Chemically and crystallographically they are essentially identical, consisting of single-crystal α-alumina or corundum. Both owe their colour to the presence of other metallic elements as impurities; in ruby, for example, the characteristic red colour is imparted by chromium which, in the form of oxide, is incorporated in the aluminium oxide structure. In the absence of impurity elements sapphire is a colourless or "white" crystal.

Blue, red (ruby) and "white" sapphire have all been made for many years in the laboratory. Originally the intention was presumably to make available gemstones at a price much lower than the natural variety, but at a later date sapphire and ruby were made to supply industrial needs. Among the most important of these has been material for jewel bearings, manufactured for instruments, watches, etc. This use arises from the fact that corundum is extremely hard and second only to diamond on the conventional Moh's scale of hardness. Recently many new industrial applications have arisen for most of which the colourless or "white" sapphire is the material required.

The most successful and most common method for the production of these synthetic crystals is the flame-fusion process developed by Verneuil about 1904.[1,2] A large single crystal or boule, probably weighing 50 to 70 g, is the first product of the Verneuil process, and this boule can then be cut and worked to give material of the dimensions and shape required for the intended industrial use. For production of the coloured crystals the appropriate impurity is added to the aluminium oxide powder that serves as the "raw material" of the flame-fusion process; however, much of this additive, together with other accidental impurities, is driven off at the high temperature attained in the process (about 2000° C). Some degree of inhomogeneity of impurity distribution may in fact occur in the boule single crystal.

A recent and most important application of synthetic ruby is in the preparation of maser crystals. The maser is a device for microwave amplification by stimulated emission of radiation, and it has been found that the chemical and crystallographic nature of ruby is particularly suited to a special version of this device known as the laser, in which the microwaves are replaced by optical radiation in the visible or near-visible range. One of the problems in maser studies on ruby, and in related work on "white" sapphire, is to investigate the relationship between the microwave spectral data and the nature, amount and distribution of impurities in the crystals. It is desirable for this purpose to have detailed analytical information concerning the concentration and distribution of added and accidental impurities down to the lowest detectable level.

Bearing in mind the refractory nature of sapphire and ruby, particularly in the form of a massive piece of single crystal, the analytical problem is considerable. The materials are difficult to decompose except by certain fusion methods, for which the sample must be in a finely crushed form. The extreme hardness of the materials creates special problems in the preparation of an uncontaminated finely crushed sample, particularly for the determination down to low levels of an impurity such as iron.

In the sections below are described the analytical investigations by means of which methods have been established for determining chromium, an added impurity, and iron, generally a fortuitous impurity, in single-crystal material. These methods, both chemical and spectrographic, relate to the determination of the average or total amount of chromium or iron in the whole of the sample selected for examination. In addition, an account is given of appropriate methods of sampling used to make a preliminary assessment of the distribution of chromium in ruby boule.

THE ANALYTICAL PROBLEM

CHROMIUM IN RUBY—

Ruby crystals of different chromium contents, representing the range of colours normally used for gems or jewel bearings, are available, and it has been possible to explore the range of chromium contents in which maser action is most prominent. This has shown the need, in work on maser crystals, for analytical methods to cover the range 0·01 to 0·5 per cent. of chromium. To permit analysis of small samples, *e.g.*, in distribution studies, it was required that the method should be applicable to sample weights of about 10 mg, *i.e.*, corresponding to the determination of 1 to 50 μg of chromium. Suitable methods of measurement, both chemical and spectrographic, are, of course, available for these amounts of chromium. For the chemical determination the method adopted was decomposition of the crushed sample by fusion and subsequent development of the chromium - diphenylcarbazide colour, which lends itself to precise spectrophotometric measurement. While corrections for the blank value are essential in this method, the amounts of chromium likely to be introduced from reagents, etc., are not sufficiently large to endanger the accuracy of the determination. The real difficulty is associated with the sampling and preparation of material of such considerable hardness and of the geometrical shape of the Verneuil boule; particular care is needed to avoid the introduction of extraneous chromium during the crushing process.

IRON IN SAPPHIRE AND RUBY—

Most of the analytical interest in iron has been concerned with the determination of the amounts present as an adventitious impurity in "white" sapphire and in ruby; these amounts, which are small compared with the chromium content of ruby, fall in the range 0·002 to 0·01 per cent. Consideration was again given to methods applicable to small weights of sample, of about 20 mg, corresponding to the determination of 0·4 to 2 μg of iron. Some determinations have been required on experimental materials having deliberate additions of iron, but even here the levels (up to 0·2 per cent.) have been lower than those mentioned in connection with chromium. Both chemical and spectrographic methods have again been used.

In the determination of iron, the problems of sample preparation are more severe than for the chromium determination. The risk of contamination with the element to be determined is much greater, and the levels to be determined are lower. For chemical determination of iron, reagents of sufficient sensitivity are available, but further problems can arise. The necessary fusion stage provides an opportunity for an exchange of iron between melt and crucible material; in addition, reagents of normal analytical purity may bring into the determination amounts of iron which in total may be as much or more than the amount of iron it is required to measure. On the other hand, a direct spectrographic technique enables the analyst to avoid these chemical difficulties, though not those of the sampling process.

THE DETERMINATION OF CHROMIUM IN RUBY

SAMPLING—

One satisfactory method involves the initial reduction of the ruby sample to a coarse powder by thermal shock. Pieces of boule, held in platinum-tipped tongs, are heated to redness in a flame and dropped into water. The resulting coarse powder is then further crushed in a mortar made from either steel or tungsten of high purity; the crushed material is sifted through a 60-mesh silk or nylon screen for the spectrographic method or through a 120-mesh screen for the chemical method; oversize material is again crushed and sifted. The powdered sample is treated with hydrofluoric and nitric acids to remove any tungsten derived from the mortar, or is boiled for a few minutes with aqua regia if a steel mortar has been used. Finally the acid-washed powder is washed three times by decantation with de-ionised water and dried at 110° C for 30 minutes.

When the determination of both iron and chromium is required on the same prepared sample, use of the tungsten mortar is essential (see section on "Determination of Iron"—"Sampling").

When knowledge about the local concentration of chromium is required a different method of sampling is employed. The boule is trepanned at each sampling point, to a depth of 3 to 4 mm, with a tool made from 16 s.w.g. steel tube. This tool is attached to a Mullard ultrasonic 60-watt drill unit; boron carbide (300 mesh) is used as the abrasive. Each cylindrical piece cut out by this technique weighs 7 to 10 mg. Each piece is crushed in a steel percussion mortar, and the resulting powder is sifted, acid-cleaned, washed and dried as described above.

CHEMICAL METHOD

The method adopted involves fusion of the prepared ruby sample in platinum with a sodium carbonate - sodium tetraborate mixture; this is in our experience the most suitable method of attack on sapphire and ruby materials. The determination is completed spectrophotometrically by a conventional diphenylcarbazide method.

REAGENTS—

Standard potassium dichromate stock solution—Dissolve 0·707 g of analytical-reagent grade potassium dichromate in water, and dilute to 1 litre.

1 ml ≡ 250 µg of chromium.

Standard potassium dichromate working solution—Prepare when required for use by diluting 1 ml of the stock solution to 100 ml with water.

1 ml ≡ 2·5 µg of chromium.

Sodium carbonate - sodium tetraborate flux—Mix intimately 10 g of analytical-reagent grade anhydrous sodium carbonate with 5 g of anhydrous sodium tetraborate (prepared by ignition of the hydrated analytical-reagent grade salt).

Aluminium potassium sulphate solution—Dissolve 1 g of analytical-reagent grade hydrated aluminium potassium sulphate in 100 ml of water.

1 ml ≡ approximately 1 mg of alumina.

Sulphuric acid, 0·4 N—Dilute 400 ml of N sulphuric acid with water to 1 litre.

Sulphur dioxide solution—Prepare a saturated solution of the gas in water.

Potassium permanganate, approximately 0·1 N—Dissolve 0·32 g of analytical-reagent grade potassium permanganate in 100 ml of water.

Sodium azide solution—Dissolve 1 g of sodium azide in 100 ml of water.

Diphenylcarbazide solution—Dissolve 0·25 g of analytical-reagent grade diphenylcarbazide in 100 ml of analytical-reagent grade acetone. Prepare fresh each day.

CALIBRATION—

In each of six 100-ml beakers place 0·1 g of flux, 10 ml of aluminium potassium sulphate solution and 25 ml of 0·4 N sulphuric acid. To the contents of the separate beakers add from a burette amounts of 0, 1, 2, 3, 4 and 5 ml of the working standard dichromate solution (1 ml ≡ 2·5 µg of chromium). Treat each solution as described below.

Add 2 drops of sulphur dioxide solution to reduce the dichromate, and boil the solution vigorously for 1 to 2 minutes to remove excess of sulphur dioxide; then add 3 drops of 0·1 N potassium permanganate, and continue boiling for 5 minutes to re-oxidise the chromium to the sexavalent state.

Cool somewhat, then add sodium azide solution dropwise to de-colorise the solution. Avoid using a large excess; 5 to 10 drops is usually adequate. Boil the solution for 1 to 2 minutes to remove hydrazoic acid, cool thoroughly, then transfer to a 50-ml calibrated flask. Add 5 ml of diphenylcarbazide solution, dilute to the mark with water, and mix the solution.

Allow 5 to 10 minutes to elapse for full colour development, and then measure the optical density of each solution at 540 mμ in a 4-cm cell, with water in the reference cell. Deduct the reading for the solution containing no added chromium from the readings for the other solutions. From these results prepare a calibration graph or calculate the mean factor relating corrected optical density to micrograms of chromium.

PROCEDURE—

On a semi-micro balance weigh out 2 to 10 mg of the prepared sample, depending on the expected chromium content. (For chromium contents up to 0·1 per cent. use approximately 10 mg of sample, for contents of 0·1 to 0·2 per cent. use approximately 5 mg and for contents of 0·2 to 0·5 per cent. use approximately 2 mg of sample.) Transfer the weighed sample portion to a small platinum crucible of about 5 ml capacity, add 0·1 g of flux, and mix throughly with a glass rod.

Heat the covered crucible over a Meker burner, gently at first, then strongly for 10 minutes. Swirl the crucible occasionally to aid attack of the sample.

Cool, then transfer the crucible and lid to a 100-ml beaker, add 25 ml of 0·4 N sulphuric acid, and heat on a hot-plate until the melt has dissolved; complete dissolution should be obtained.

Remove the crucible and lid, rinse them, add the rinsings to the solution, and heat the solution to boiling. Add 3 drops of 0·1 N potassium permanganate, and continue boiling for 5 minutes. Complete the procedure for development and measurement of the chromium colour as described under "Calibration."

Run a blank determination in parallel with the sample determination, and correct for any chromium found. Finally, calculate the chromium content of the sample.

SPECTROGRAPHIC METHOD

For the spectrographic determination of chromium, the prepared sample is mixed with a carbon-powder spectrographic buffer containing cobalt as the internal standard. The mixture is placed in a cupped graphite electrode, and the chromium is determined by a d.c. arc total-combustion technique. Standardisation is effected by comparison with mixtures prepared from ruby in which the chromium has been determined chemically.

The same spectrographic buffer and arc conditions are also used in the determination of iron; if required, chromium and iron can be determined simultaneously, provided that the method of sample preparation has been appropriate for determining iron. For convenience, details of the spectrographic procedure relating to the determination of iron are given separately under "Determination of Iron."

In experiments on the spectrographic method, particular attention was paid to assessing the effect of particle size of the sample and to observing the variation with time of the evaporation of chromium and cobalt (and also iron). No difference in evaporation behaviour was observed between samples and standards. The use of a sample of different particle size showed that material of less than 60 mesh was satisfactory, no difference being observable with more finely crushed material.

APPARATUS—

Spectrograph—Hilger large-quartz spectrograph with a mask containing a 5-mm horizontal slit adjacent to the plate.

Excitation source—A d.c. arc source capable of providing a current of 15 amps; open circuit voltage at least 120 volts.

Slit height—2 mm.

Slit width—0·015 mm.

External optics—A 30-cm quartz lens, placed adjacent to the slit so as to form an image of the arc on the collimating lens of the spectrograph.

Wavelength range—3400 to 2450 Å.

Plate calibrator—A rotating sector disc with a step-to-step ratio of 2 to 1.

Densitometer—Hilger non-recording densitometer.

Electrodes—Upper electrode (negative): a 1 inch length of 6·35-mm diameter Ringsdorff RW-O graphite rod. Lower electrode (positive): Ringsdorff RW0078 electrode.

Photographic plates—Kodak B.10.

REAGENTS—

Crushed ruby boule—Ruby boule crushed as described under "Sampling" and of known chromium content as determined by the chemical method.

Crushed sapphire boule—"White" (*i.e.*, chromium-free) sapphire boule crushed as described under "Sampling."

Spectrographic buffer—Mix 9·72 g of carbon powder, prepared by turning JMIB carbon rods, with 0·28 g of cobalt oxide in an agate mortar.

Developer—Dilute 1 part of Ilford ID2 stock solution with 2 parts of water.

PREPARATION OF CHROMIUM STANDARDS—

Prepare in duplicate a series of standards, covering the range 0·005 to 0·25 per cent. of chromium, as described below.

Weigh out 5·0 mg of crushed, chemically analysed ruby boule, or appropriate portions of chemically analysed ruby and "white" sapphire to give a total of 5·0 mg. Mix with 5·0 mg of spectrographic buffer in an agate mortar, and transfer to an electrode.

Record the spectra of the standards as described below.

PROCEDURE—

Weigh out 5·0 mg of the prepared sample, mix in an agate mortar with 5·0 mg of spectrographic buffer, and transfer to an electrode.

Place a pair of electrodes in the arc stand with the filled one in the lower (positive) arm. Touch the electrodes together, switch on the current, separate the electrodes to give a 4-mm gap, and adjust the control rheostat to give a current of 10 amps. Run the arc for 2 minutes, maintaining an electrode separation of 4 mm.

To record a calibrating spectrum on the same plate, remove the mask, increase the slit height to 12 mm, place the sector disc in position, and photograph a 5-amp iron arc for 60 seconds.

Develop the plate for 3 minutes at 20° C, fix, wash and dry.

Measure the transmission of the chromium line at 2672·83 Å and the cobalt line at 2650·27 Å, and of the spectral background adjacent to each line.

CALCULATION OF RESULTS—

Construct a plate calibration curve from the transmission readings from the calibrating spectrum. Convert the transmission readings for the chromium and cobalt lines to relative intensity, and correct the line intensities for background intensities. Calculate the ratio of the chromium and cobalt line intensities, and convert this to percentage chromium by reference to a working curve.

The working curve is prepared on log - log paper from the spectra of the standards.

RESULTS

When ruby samples were analysed for chromium both chemically and spectrographically, satisfactory agreement between the methods was obtained. This is illustrated in Table I, which shows values obtained by both methods on samples covering a range of chromium contents.

A typical experiment to assess the distribution of chromium in a "flat" ruby boule is illustrated in Fig. 1. The samples for analysis were removed from the flat face of the boule, at the points shown, by the trepanning technique previously described under "Sampling"; the chromium contents were determined chemically. As shown in Fig. 1, the results obtained covered the range 0·016 to 0·021 per cent. of chromium, although the alumina powder from which the boule was grown contained an addition corresponding to 0·1 per cent. of chromium. This illustrates the large loss of additive that can occur in the flame-fusion process.

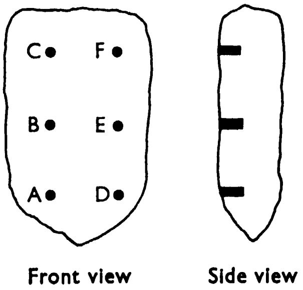

Fig. 1. Distribution of chromium in a flat ruby boule. Percentage of chromium found at sampling points: A, 0·021; B, 0·021; C, 0·021; D, 0·017; E, 0·016; F, 0·016

TABLE I

COMPARISON OF CHEMICAL AND SPECTROGRAPHIC RESULTS FOR CHROMIUM IN RUBY

Sample number	Chromium content found by— Chemical method, %	Spectrographic method, %
26777	0·015	0·016
26707	0·058	0·053
27153	0·10	0·11
27154	0·20	0·19

THE DETERMINATION OF IRON IN SAPPHIRE AND RUBY

SAMPLING—

Extreme care is required to avoid the introduction of extraneous iron during the preparation of the sample. The method described below was found to be satisfactory.

Heat the sample piece, held in platinum-tipped tongs, to red heat in a flame, and then drop into "pure" water to shatter the sample by thermal shock. Pour off the water, dry the sample at 110° C, and then transfer to a mortar made from tungsten of high purity and provided with a well-fitting tungsten rod as a pestle. Fit a polythene cap to the upper end of the pestle, and tap this gently with a hammer to crush the sample. Sift the fine material through a 60-mesh silk or nylon screen for the spectrographic method or through a 120-mesh screen for the chemical method. Return the oversize material to the mortar, and again crush so that all the material passes through the screen.

Transfer the sifted powder to a clean platinum crucible, add 2 ml of hydrofluoric acid and 2 ml of nitric acid, and warm the mixture for about 5 minutes to dissolve any tungsten acquired in the crushing. Dilute with water, allow the powder to settle, and carefully decant off the liquid. Wash the powder with water by decantation several times, dry at 110° C, and store the dried powder in a clean capped specimen-tube.

SPECTROGRAPHIC METHOD

In this particular context, a spectrographic determination offers great advantages over a chemical method. The relatively high blank values for iron resulting from reagents of normal purity (and the consequent need in a chemical method to purify the reagents) are eliminated by the use of a direct spectrographic technique. It was established by experiment that the presence of chromium had no effect on the iron spectrum, so that the same technique was applicable to the determination of iron in colourless sapphire and in ruby.

Although it has been shown that the iron content of ruby and sapphire is generally in the region of 0·005 per cent., the method described below has been devised for iron contents up to 0·2 per cent.; this has permitted the analysis of experimental material containing deliberately added iron. As stated earlier, the spectrographic buffer and arc conditions used are the same as for the chromium determination, so that iron and chromium can be determined simultaneously if required.

APPARATUS AND REAGENTS—

The apparatus, spectrographic buffer, electrodes, photographic plates and developer are as described for the determination of chromium. Other reagents required specifically for the determination of iron are listed below.

Crushed boule—Ruby or sapphire boule of low iron content, crushed as described under "Sampling."

Aluminium ammonium sulphate, hydrated—Analytical-reagent grade.

Ammonium ferric sulphate, hydrated—Analytical-reagent grade.

Ammonia solution, sp.gr. 0·88—Analytical-reagent grade.

Wash solution—A 2 per cent. w/v solution of ammonium nitrate made just alkaline to methyl red by adding ammonia solution.

PREPARATION OF STOCK ALUMINA - IRON MIXTURES FOR IRON STANDARDS—

Prepare two stock mixtures of alumina, containing 0·1 and 1 per cent. of iron, as described below.

For the 0·1 per cent. mixture, weigh out and transfer to a 250-ml beaker 8·88 g of aluminium ammonium sulphate and 0·0086 g of ammonium ferric sulphate. For the 1 per cent. mixture weigh out and transfer to a second beaker 8·80 g of aluminium ammonium sulphate and 0·0861 g of ammonium ferric sulphate.

Dissolve the salts in water, and dilute to about 150 ml. Add 2 or 3 drops of methyl red indicator, heat the solution to boiling, and add ammonia solution until the indicator just turns yellow. Set the solution aside on a water-bath for 10 minutes.

Filter off the precipitate on a Whatman No. 41 or equivalent filter-paper, and wash the precipitate with hot wash solution. Transfer the paper and precipitate to a silica crucible, dry, and then ignite at a low heat until all carbonaceous matter has been removed. Finally, heat the crucible and contents in a muffle furnace at 1000° C for 1 hour. Cool, transfer the mixture to an agate mortar, and grind to a fine powder.

PREPARATION OF IRON STANDARDS—

Prepare in duplicate a series of standards, covering the range up to 0·2 per cent. of iron, as described below.

Weigh out 5·0 mg of spectrographic buffer, add the weight of alumina - iron mixture specified in Table II, and mix in an agate mortar. Add 5·0 mg of crushed boule, mix in the mortar, and transfer the mixture to an electrode.

Record the spectra of the standards as described below.

TABLE II

WEIGHTS OF ALUMINA - IRON MIXTURES FOR SPECTROGRAPHIC IRON STANDARDS

Iron content of standard, %	Weight of alumina - iron mixture added
Blank	—
0·006	0·30 mg of 0·1% mixture
0·02	1·00 mg of 0·1% mixture
0·06	0·30 mg of 1% mixture
0·20	1·00 mg of 1% mixture

PROCEDURE—

Weigh out 5·0 mg of the prepared sample, mix in an agate mortar with 5·0 mg of spectrographic buffer, and transfer to an electrode.

Excite the sample, and record the spectrum: also record a calibrating iron spectrum on the same plate, exactly as described for chromium. Develop the plate for 3 minutes at 20° C, fix, wash, and dry.

Measure the transmission of the iron lines at 2611·87 and 2621·68 Å and of the cobalt line at 2590·59 Å, and of the spectral background adjacent to each line.

CALCULATION OF RESULTS—

Construct a plate calibration curve from the transmission readings for the calibrating spectrum. Convert the transmission readings for the iron and cobalt lines to relative intensity and correct the line intensities for background intensities. Calculate the ratio of iron and cobalt line intensities and convert this to percentage iron by reference to a working curve.

The working curve is prepared on log - log paper from the spectra of the standards. For this purpose deduce from the spectra, by the method of standard additions,[3] the iron content of the crushed boule used in the standards, and correct the nominal iron content of each standard accordingly.

CHEMICAL METHOD

As described above, the preparation of standards in the spectrographic technique involves the mixing of solid materials, a crushed sapphire or ruby being "doped" with alumina - iron mixtures of known iron content. Excellent results over a range of iron contents, *e.g.*, with materials containing deliberate additions of iron, were obtained by the use of these synthetic standards. However, a possible doubt attached to the spectrographic determination of iron at the lowest levels, *e.g.*, in the purest undoped materials, as this necessarily involved an extrapolation of the working curve.

For this and other reasons, a considerable effort was put into the development of a chemical method suitable for determining iron at the lowest levels. Spectrophotometric determination with bathophenanthroline[4] was used; this is the most sensitive and selective reagent available for iron, and there was no difficulty in achieving the required sensitivity. In the development of the method, the two aspects requiring most consideration (apart from the sampling problem already discussed) were the method of decomposition and the need to attain adequately low blank values. These two points are discussed in more detail below.

DECOMPOSITION—

As in the determination of chromium, the most suitable method of decomposition involves a fusion process for attack of the sapphire or ruby. Although there are no special problems with chromium, the choice of flux and of crucible material are all-important in the determination of iron. In particular, there is a need to minimise the possibility of exchange of iron between crucible and melt (and of attack on the crucible), and it is desirable to achieve complete decomposition in the shortest possible time. After an investigation of various fluxes and crucible materials, the process finally adopted involved "sintering" in platinum at 1050° C with a small portion of flux consisting of anhydrous sodium carbonate and anhydrous sodium tetraborate in the proportion 2 to 1 by weight. Only 50 mg of flux and 20 mg of sample were used, and the mix was heated in a platinum crucible in an electric muffle furnace.

The "sinter" process was introduced by Finn and Klekotka of the National Bureau of Standards in 1930,[5] and this technique has been in use in these laboratories for many years for decomposing glass and refractory materials. In the present context, the sinter technique has several advantages—

(*i*) the process is quick; complete attack of the sample is obtained within 10 minutes' heating;

(*ii*) the small amount of flux reduces the blank value arising from iron introduced from this source;

(*iii*) both constituents of the flux are readily re-crystallised and therefore potentially amenable to purification;

(*iv*) while it is known that fusions in platinum can give rise to exchange of iron between melt and crucible, the use of a sinter rather than a fluid melt minimises the extent of this exchange process.

It has nevertheless been thought an essential precaution to do one or more blank fusions in the crucible immediately before and after each determination of iron. If results at the customary blank level are obtained, this provides an assurance that exchange of iron with the crucible has not occurred in the determination. As is our normal practice when determining small amounts of iron, a platinum crucible was reserved solely for the work on sapphire and ruby.

BLANK VALUES—

In trace analysis, the contribution from reagents and apparatus of the element to be determined should ideally be appreciably less than the amount in the sample. It may be desirable, as in our work, to adopt an analytical process permitting the use of reagents that either are available in a high state of purity or readily lend themselves to purification.

In the method devised for determining iron, the reagents used are: sodium carbonate - sodium tetraborate flux, hydrochloric acid, a combined reducing and buffer solution containing hydroxylammonium chloride and sodium acetate, bathophenanthroline solution and chloroform. In initial work on this method, only the reducing - buffer solution was purified (by development and extraction of the iron - bathophenanthroline colour). The blank values were at the level of 0·7 to 0·8 μg of iron, *i.e.*, similar to and sometimes greater than the amount of iron in a sample. Subsequently, the blank value was substantially reduced by decreasing the amount of bathophenanthroline used, by employing distilled hydrochloric acid and by purifying the constituents of the flux. Consistent blank values at the level of 0·2 μg of iron were then obtained, an amount equivalent to only a half of the smallest amount of iron determined in a sample.

For purification of the constituents of the flux, use was made of the reagent phenyl-2-pyridyl ketoxime, which has the unusual property of forming an extractable complex with ferrous iron in strongly alkaline solutions.[6,7] Separate strong aqueous solutions of sodium carbonate and of sodium tetraborate were prepared, iron was extracted as the phenyl-2-pyridyl ketoxime complex, and the sodium carbonate and tetraborate were re-crystallised from solution. Purification in this manner was more successful with sodium tetraborate than with sodium carbonate, a possible explanation being the presence of iron in an insoluble form in the sodium carbonate. Better results with the sodium carbonate were achieved by introducing a filtration step, with titanium to serve as a "collector," before the extraction.

Experimental details of the chemical method for determining iron in sapphire and ruby are given below.

PREPARATION AND PURIFICATION OF REAGENTS—

Water—Use distilled water further purified by passage through mixed-bed ion-exchange resin. Store in a polythene container.

Hydrochloric acid—Purify by isopiestic distillation[8] as described below. Clean a 10-inch vacuum desiccator with hydrochloric acid, dry the desiccator, and remove any lubricant. Into the base of the desiccator pour 500 ml of analytical-reagent grade hydrochloric acid, sp.gr. 1·18. To a polythene or polystyrene container add 100 ml of water, and support the container on glass rods above the level of the acid; close the desiccator with the lid. At daily intervals withdraw a small aliquot from the solution in the plastic container, making use of the hole in the desiccator lid, and by titration determine the strength of acid. Continue the distillation until acid of 8 N concentration is obtained (2 to 3 days). Store the distilled acid in a glass-stoppered bottle.

Bathophenanthroline solution—Dissolve 0·0668 g of bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) in 100 ml of analytical-reagent grade ethanol, and dilute to 200 ml with water. Store in a glass-stoppered bottle.

Chloroform—Analytical-reagent grade.

Reducing — buffer solution—Dissolve 20 g of analytical-reagent grade sodium acetate trihydrate and 2·0 g of analytical-reagent grade hydroxylammonium chloride in water, transfer to a separating funnel, and dilute to 200 ml. Add 2 ml of bathophenanthroline solution, mix, and set aside for 1 minute. Add 10 ml of chloroform, shake vigorously for 1 minute, allow the layers to settle, and run off the chloroform layer. Repeat the extraction, adding further bathophenanthroline solution, if necessary, until a colourless extract is obtained. Store the purified solution in a glass-stoppered bottle.

Sodium carbonate—Dissolve 10 g of analytical-reagent grade anhydrous sodium carbonate in 50 ml of hot water, and add a solution containing the equivalent of about 5 mg of titanium dioxide (prepared by dissolving 25 mg of potassium titanyl oxalate in a few drops of sulphuric acid (1 + 1), heating to fumes, then cooling and diluting with about 5 ml of water). Digest the solution at about 70° C for 30 minutes, and then filter through a coarse paper, without washing the precipitate. Re-heat the filtrate to about 70° C, add 0·2 g of sodium dithionite and 5 ml of phenyl-2-pyridyl ketoxime solution (0·2 g dissolved in 100 ml of 0·1 N hydrochloric acid), and keep the solution hot for about 5 minutes. Cool the solution, transfer to a separating funnel, and extract with successive 10-ml portions of chloroform until a colourless extract is obtained (2 or 3 extractions should suffice). Run the aqueous solution into a beaker, heat to 50° C, add 50 ml of ethanol, and cool. Pour off the alcohol layer, again heat to 50° C, add 25 ml of ethanol, cool, and discard the alcohol layer; if necessary, repeat the heating, the addition of 25 ml of ethanol and the cooling to obtain crystallisation of sodium carbonate from the aqueous solution. Filter off the crystals on a No. 4 grade sintered-glass crucible, and wash once with ethanol. Transfer the crystals to a clean platinum crucible, dry at 110° C then heat at 250° C to convert the precipitated monohydrate into anhydrous sodium carbonate. Store in a screw-capped bottle.

Sodium tetraborate—Dissolve 20 g of analytical-reagent grade sodium tetraborate in 100 ml of hot water. Add 0·2 g of sodium dithionite and 5 ml of phenyl-2-pyridyl ketoxime solution, and maintain the solution at about 70° C for 5 minutes. Cool the solution to about 40° C, transfer to a separating funnel, and extract the solution with two successive 10-ml portions of chloroform, rejecting the extracts; complete the extractions quickly so as to minimise risk of crystallisation of the sodium tetraborate. Pour the aqueous solution into a beaker, and cool the solution, stirring during the cooling to prevent the crystals adhering to the walls of the beaker. Filter off the crystals on a No. 4 sintered-glass crucible, and wash once with ethanol. Transfer the crystals to a clean platinum crucible, dry at 110° C, and then heat at 300° C to dehydrate the sodium tetraborate. Store in a screw-capped bottle.

Flux—Mix purified sodium carbonate and purified sodium tetraborate in the proportions 2 to 1 by weight. Store in a screw-capped bottle.

Standard iron stock solution—Dissolve 0·100 g of pure iron in a few millilitres of dilute sulphuric acid (1 + 6), and dilute with water to 1 litre.

$$1 \text{ ml} \equiv 100\ \mu\text{g of iron.}$$

Standard iron working solution—Prepare when required for use by diluting 1 ml of the stock solution to 100 ml with water.

$$1 \text{ ml} \equiv 1\ \mu\text{g of iron.}$$

CALIBRATION—

To each of four dry 50-ml separating funnels add 2 ml of water, 0·2 ml of hydrochloric acid and 50 mg of flux. To the solutions in three of the funnels add 0·5-, 1·0- and 2·0-ml portions of the working standard iron solution, corresponding to 0·5, 1·0 and 2·0 µg of iron. Dilute all four solutions to 5 ml with water, and treat each as described below.

Add 5 ml of reducing - buffer solution (the amounts of reagents used give a pH of 4·7 to 4·9 at this stage). Add 2 ml of bathophenanthroline solution, mix and set aside for 1 minute. Add from a pipette 10 ml of chloroform, shake vigorously for 1 minute, and allow the layers to settle. Run off the chloroform layer through a small roll of filter-paper in the stem of the funnel (to absorb any entrained water) into a 4-cm cell. With chloroform in the reference cell, measure the optical density of the extract at 535 mµ; use a suitable mask if the volume of solution is insufficient to fill the light path of the spectrophotometer.

From the optical densities of the solutions with added iron subtract the optical density of the solution with no added iron. From these results prepare a calibration graph or calculate the mean factor relating corrected optical density to micrograms of iron.

PROCEDURE—

To a semi-micro platinum crucible, specially reserved for the determination, add 50 mg of flux. Cover the crucible and with platinum-tipped tongs transfer it to a muffle furnace at 1050° C, taking care to handle only the outside of the crucible. Heat for 10 minutes, remove the crucible, and allow it to cool.

Add 2 ml of water and 0·2 ml of hydrochloric acid, and gently heat the covered crucible on a hot-plate for a few minutes to dissolve the fused mass. Allow the crucible to cool, transfer the solution to a dry 50-ml separating funnel, and dilute with water to 5 ml. Add 5 ml of the reducing - buffer solution and 2 ml of bathophenanthroline solution, set aside for 1 minute, and then proceed with the extraction and spectrophotometric measurement of the extract as described under "Calibration." Calculate the amount of iron found.

If the amount of iron found is at the normal level of the blank value, proceed with a determination. If an excessive amount of iron is obtained, or if a crucible is being used for the first time, repeat the blank fusion as above until the amount of iron found is at a suitably low level. (With reagents purified as described, the amount of iron customarily found in blank fusions was 0·2 ± 0·05 μg of iron.)

For the determination, add 50 mg of flux and 20 mg of the prepared sample to the crucible, and mix with a clean glass rod. Cover the crucible, and heat for 10 minutes at 1050° C in a muffle furnace. Allow the crucible to cool, add 2 ml of water and 0·2 ml of hydrochloric acid, and gently heat the covered crucible on a hot-plate until dissolution of the sintered mass is complete; heating for 15 to 20 minutes may be required. Cool the crucible, transfer the solution to a separating funnel, and proceed with the development, extraction and measurement of the iron colour as previously described. Calculate the total amount of iron found.

Without delay carry out a blank fusion in the crucible and determine the amount of iron obtained. If this amount is at the customary blank level, calculate the mean blank value from the amounts of iron found for the blank fusions made immediately before and after the determination on the sample. Subtract this mean blank value from the total amount of iron found in the determination on the sample, and hence calculate the percentage of iron in the sample.

Because of the small amounts of iron to be determined, constant precautions are needed to avoid contamination of solutions and apparatus from airborne dust, etc.

RESULTS

To illustrate levels of the blank value and the actual amounts of iron commonly determined, a chemical determination is shown in detail in Table III. As will be seen from Table III, the blank fusions before and after the determination on the sample gave amounts of iron at the customary blank level of 0·2 μg of iron, thus providing an assurance that no significant exchange of iron between crucible and melt had occurred in the intervening determination. The material analysed in this particular determination was sapphire grown in these laboratories.

TABLE III

DETAILS OF A DETERMINATION OF IRON IN "WHITE" SAPPHIRE

Operation	Optical density of extract	Iron found, μg
Blank fusion before determination	0·033	0·23
Determination on 20-mg sample	0·080	0·55
Blank fusion after determination	0·028	0·19

Mean blank value = 0·21 μg of iron
Iron in sample = 0·55 − 0·21 = 0·34 μg = 0·002%

In Table IV are shown results obtained for iron by both chemical and spectrographic methods on a range of samples. The main interest in determining iron has been concerned with sapphire and ruby in which iron is present only as an adventitious impurity, and most of the results given in Table IV relate to this type of material (iron content generally 0·002 to 0·005 per cent.). Satisfactory agreement between the two methods was obtained at these levels and the chemical method, although relatively lengthy, has thus provided independent evidence that the spectrographic technique is applicable at these low levels of iron content.

An independent check by neutron-activation methods on two of the samples listed in Table IV, was arranged through Dr. C. A. Parker of the Admiralty Materials Laboratory. Results for iron were obtained by direct gamma-ray spectrometry on the irradiated sample and also by a more rigorous method in which the iron was separated chemically before being counted. The respective results by these two techniques were 0·004 and 0·001 per cent.

of iron on sapphire No. 28171 (for comparison with the figures given in Table IV, *i.e.*, mean values of 0·002 per cent. chemically and 0·002 per cent. spectrographically) and 0·002 and 0·001 per cent. on ruby No. 27154 (for comparison with figures of 0·004 per cent. chemically and 0·0045 per cent. spectrographically in Table IV). These results by activation methods, though not in precise agreement with our own values, do help to establish the general level of iron content in the sapphire and ruby materials. It should be added that in the determination of chromium (a much less exacting problem), excellent agreement was obtained between results at the Admiralty Materials Laboratory and those reported in Table I. On ruby No. 27154 results of 0·20 and 0·20 per cent. of chromium were obtained by gamma-ray spectrometry on the irradiated sample, compared with values of 0·20 and 0·19 per cent. of chromium by chemical and spectrographic methods, respectively.

To illustrate determination at higher levels, Table IV includes some results on "doped" materials, *i.e.*, with iron deliberately added during their preparation. Again, satisfactory agreement between chemical and spectrographic methods was obtained. At these higher levels, the problems of the chemical determination of iron are greatly simplified; purification of reagents becomes unnecessary and a less sensitive reagent, *e.g.*, thioglycollic acid, can be used if required.

TABLE IV

COMPARISON OF RESULTS BY CHEMICAL AND SPECTROGRAPHIC METHODS FOR IRON IN SAPPHIRE AND RUBY

Sample	Sample number	Iron content found by— spectrographic method, %	Iron content found by— chemical method, %
"Pure" materials—			
Sapphire	28171	0·0025, 0·002, 0·002, 0·0015	0·002, 0·002
Sapphire	27309	0·004, 0·004	0·003, 0·003, 0·002
Sapphire	27308	0·003, 0·003, 0·005	0·004, 0·003, 0·003
Ruby	26707	0·004, 0·004, 0·004, 0·006	0·004
Ruby	27154	0·0045 (mean value)	0·004
Materials "doped" with iron—			
Ruby	26777	0·10	0·09, 0·09
Corundum	25163	0·19, 0·22	0·18

DISCUSSION

In the analysis of sapphire and ruby by chemical or spectrographic methods, the process of sample preparation is a critical part of the procedure, particularly in the determination of iron. In the investigation of various sampling techniques, spectrographic examination has been of value in indicating the nature and degree of any contamination that might arise at the various stages in the sampling process. We are satisfied that the techniques adopted will yield prepared sample material essentially uncontaminated with the element to be determined.

In the preceding descriptions of the analytical methods, the chemical method has been given first in the section on chromium, and the spectrographic method first in the section on iron; the order chosen is both logical and chronological. For chromium in ruby, the chemical method was developed first, and chemically analysed ruby was subsequently used as a means of calibrating the spectrographic method for chromium. With the determination of iron, the obvious difficulties of a chemical method encouraged the initial development of a spectrographic method; this in turn led to the development of a chemical method as a means of assessing the validity of the spectrographic method at low levels of iron content.

The work has shown the spectrographic technique to be applicable over an appreciable range of chromium and iron contents, and this technique has been the preferred method for routine work. Once the calibrations have been completed the procedure is rapid; iron and chromium can be determined simultaneously, and the sample preparation is a little less exacting in that material of less than 60 mesh is required instead of less than 120 mesh for the chemical methods.

The chemical method for iron has served its immediate purpose of verifying the validity of the spectrographic technique at low levels of iron content and of helping to establish the levels of adventitious iron present in sapphire and ruby. It requires considerable care and is relatively time-consuming, and is now used only for the occasional confirmatory test.

On the other hand, the chemical method for chromium has been of continuing value, because it has a higher precision than the spectrographic method. It has been used, for example, in establishing the existence of small differences in chromium contents in distribution studies of the type illustrated in Fig. 1.

The analytical techniques described in this paper have fulfilled exploratory requirements to establish the levels of chromium in ruby and of iron in sapphire and ruby, and to provide some information on their distribution. Ultimately, the ideal analytical technique would be a non-destructive test capable of giving point-to-point information on the distribution of "impurities" in the actual crystal to be used in a maser device. Preliminary work on the determination of chromium suggests that both X-ray fluorescence and optical absorption spectrophotometry may be of some value in this connection; with X-ray fluorescence, however, limitations may arise on sensitivity and from the fact that only the composition of material near the surface is assessed.

When the work described in this paper was essentially complete, a method for analysing sapphire and ruby maser crystals, involving a rather different approach from our own, was reported by Dodson.[9] In this method up to 20 mg of the crushed sample is decomposed by fusion for 2 to 2½ hours with about 1 g of potassium hydroxide in a zirconium crucible. Chromium is determined spectrophotometrically with diphenylcarbazide on an aliquot of the solution of the melt. On a separate aliquot iron is determined with 2,2′-dipyridyl; because of the pale colours obtained even with concentrated solutions, the iron colour is developed either in a small volume of solution in the depression of a white tile or on a small disc of filter-paper impregnated with the reagent, and the iron is assessed by visual comparison with standards similarly prepared.

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The Extraction and Identification of Permitted Food Colouring Matters with Special Reference to the Changes Undergone During Processing and Subsequent Storage

FIRST REPORT OF THE TRACE MATERIALS (COLOURS) COMMITTEE
SET UP BY THE
BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

THE Trace Materials (Colours) Committee of the British Food Manufacturing Industries Research Association was set up in April 1959 under the Chairmanship of Dr. A. A. Houghton with the following terms of reference—

> "The Committee exists to examine any problems which may arise in the use of coal-tar colouring matters in foodstuffs as a result of the Colouring Matters in Foods Regulations.
>
> The principal interest is in the effects of processing the colours in foodstuffs and of storage of the finished products. Particularly in the formation of new coloured products and the changing of the proportion of subsidiary dyes.
>
> Inevitably this involves the consideration of the best processes for isolating colours from foods, identifying them and estimating their concentration.
>
> Changes in the percentage of subsidiary dyes are important in view of the standards now being laid down by the British Standards Institution."

The present working membership of the Committee is—Mr. A. G. Sansome (Edward Sharp and Sons Ltd.), *Chairman*; Mr. N. R. Jones (B.F.M.I.R.A.), *Secretary*; Dr. D. Dickinson, assisted by Mr. T. W. Raven (Fruit and Vegetable Canning and Quick Freezing Research Association), Messrs. K. J. Gardner (Mars Ltd.), R. A. Knight (British Baking Industries Research Association), F. J. Lipscomb (John Mackintosh and Sons Ltd.), R. C. Spalding (Kent County Council Public Analysts Department) and E. F. Williams, assisted by H. C. Hornsey (J. Sainsbury Ltd.). Dr. A. A. Houghton (Mars Ltd.) and Dr. A. G. Lipscomb (John Mackintosh and Sons Ltd.) previously served on the Committee as Chairmen.

Changes undergone by food colours during processing and subsequent storage are important from two major aspects: (1) Fading or undesirable changes in shade may be such as to affect the acceptability to the consumer if the desired appearance cannot be achieved or is lost during storage. (2) The Colouring Matters in Foods Regulations permit only certain colours to be used. If the colours undergo changes resulting in the production of new coloured substances, it might be concluded that non-permitted colours had been added to the food in the first place.

Also, should the situation eventually arise wherein food colours are required to conform to the appropriate British Standards (as is envisaged), it might happen that foodstuffs would be examined to ascertain whether or not the colouring matters in them were acceptable. In particular, the proportion of subsidiary dyes might be determined, and, therefore, changes in the proportions of the main dyes and any subsidiaries are of interest, as well as the appearance of dyes not present in the original colouring matter.

To obtain this information, it was considered vital that the methods used should remove all the colour from the foodstuff, and permit it to be recovered for subsequent examination. It was considered to be particularly important that the procedures involved should avoid any severe heat treatment or the use of strong acids or alkalis, as any changes in the dyes during extraction would invalidate the conclusions drawn. The traditional wool-dyeing technique was thought to be undesirable, as repeated boiling is required to obtain complete recovery of the dyes.

METHODS

Only the methods that have been employed in the work of the Committee will be described here, and only water-soluble dyes have been investigated.

All methods may be divided into three separate parts—

(*a*) transfer of the dyes from the foodstuff into solution,
(*b*) separation of the dye from extraneous substances and
(*c*) separation of individual dyes and identification.

These parts can be regarded as unit operations that can be combined together to suit particular products and particular colouring matters.

The Committee has examined the following products as representative of different types: boiled sweets, toffees, fondant, madeira cake, fresh sausages and fish paste.

So far, these products have been coloured with the food colours listed below, but for a few of these products the whole range of colours was not used—

Tartrazine
Sunset Yellow
Amaranth
Ponceau 4R
Carmoisine
Green S
Red 10B
Red 2G
Ponceau MX
Ponceau SX
Red 6B
Black PN
Orange G
Yellow 2G
Blue VRS
Indigo Carmine
Erythrosine

In addition, Tartrazine was processed in canned peas.

The choice of dyestuffs was conditioned by the setting up of specifications by the British Standards Institution (B.S.I.), which gave standards of purity for the dyestuffs and suggested suitable solvent systems for separating subsidiary dyes from the principal colouring matters by paper chromatography. The dyes used were all of good commercial foodstuffs quality, and, where B.S.I. specifications had been published, they conformed to these specifications.

TRANSFER OF DYES FROM THE FOODSTUFFS INTO SOLUTION

Methods for extracting dyes from foods are described in the Association of Public Analysts (A.P.A.) Handbook[1] and in other places. These methods have proved quite satisfactory in practice, and it is necessary to give only a brief indication of the method used for most products.

Boiled Sweets and fondants—Direct dissolution in water was sufficient.

Toffees— A solution in water was made just alkaline with ammonia solution and filtered by suction, a large excess of filter-aid (*e.g.*, Hyflo Supercel) being used.

Madeira cake—Air-dried, and de-fatted with light petroleum before extracting the colour by soaking for several hours or overnight with cold 50 per cent. aqueous alcohol containing 1 per cent of ammonia solution, sp.gr. 0·88. Solutions were clarified by centrifugation, and the extractions were repeated when necessary. The final extracts were combined and concentrated.

Sausages—Extracted in ammoniacal 50 per cent. aqueous alcohol (20 g of sausages in 14 ml of water, 25 ml of ethanol and 1 ml of ammonia solution, sp. gr. 0·88) by setting the mixture aside in the cold for half an hour. The solution was then filtered and concentrated.

Fish paste—Extracted in ammoniacal 50 per cent. acetone (20 g of fish paste in 6 ml of water, 20 ml of acetone and 1 drop of ammonia solution, sp.gr. 0·88). After having been stirred for 10 minutes the solution was spun in a centrifuge and the acetone boiled off. Further extractions of the residues were often required to remove the whole of the colour. Fat was then removed by shaking with light petroleum.

Canned Peas—The samples were macerated in their own liquor. Colour was extracted directly from this macerate by a solvent.

In all these methods heating was kept to a minimum to avoid changes in the dyestuffs. In particular, the solutions should be concentrated under reduced pressure. Some of the methods used eliminated the need for concentration by evaporation.

SEPARATION OF DYES FROM EXTRANEOUS SUBSTANCES

The principles involved were those of solvent extraction, ion exchange and column chromatography. None of these methods is specific for synthetic dyes, nor is the wool-dyeing technique. It is always desirable to purify the first extract, either by repeating the process used or by using a different principle. The latter would seem most desirable as the impurities carried over in, for example, solvent extraction may not be carried over in a different process, such as ion exchange.

SOLVENT EXTRACTION—

The higher alcohols, particularly isobutanol and n-butanol, were the most useful of the solvents investigated. Dyes were normally extracted from aqueous solution under acid conditions and removed from the solvent by weak ammonia solution. Typical working conditions were those for canned peas—

> Macerate the peas with their own liquor. To 25 ml in a 50-ml stoppered cylinder add 2 ml of 25 per cent. w/v sulphuric acid. Warm to 50 to 60° C in a water-bath, add 15 ml of isobutanol, and shake vigorously. Decant off the isobutanol extract. Repeat until extracts contain no further colours. Combine the extracts.

The colour can be removed from this extract by direct chromatography on an alumina column or can be extracted into dilute ammonia solution. In the latter instance, ammonium sulphate will be present so that further purification by another method is desirable.

Similar methods were used successfully on sausages and madeira cake (prepared as described above) and, by using n-butanol, on boiled sweets and fish paste.

A method involving solvent extraction after formation of a complex with a quaternary ammonium compound was described by Drevon and Laur.[2] This method had the disadvantage that the complex needed to be broken down by the use of strong acids and high temperatures.

A modified method, however, has been found successful, although it has not yet been tried with all the colours examined. In this modified method a cetylpyridinium salt is used to form the complex, which is then broken down in the cold by careful neutralisation with a dilute solution of Teepol, *e.g.*, the procedure for toffees—

> Make the coloured syrup alkaline with ammonia solution, add Supercel, and filter; 25 ml of syrup should contain approximately 0·2 mg of colour. Adjust the pH to 9, add 10 ml of 0·1 per cent. cetylpyridinium chloride solution, and extract with 10 ml of chloroform. Repeat with a second 10-ml portion of chloroform, which should leave the aqueous phase colourless. Shake the chloroform extracts with successive 5-ml portions of 0·1 per cent. Teepol solution, rejecting the aqueous fractions until some colour is extracted. Retain this fraction in contact with the chloroform, and add 1 per cent. Teepol solution dropwise, shaking between additions, until no colour remains in the chloroform layer. Reject the chloroform. The aqueous layer contains a precipitate. Remove this precipitate by extracting twice with 10 ml of light petroleum (boiling-range, 40° to 60° C). Concentrate the clear aqueous dye solution under reduced pressure.

With some colours, *e.g.*, Yellow 2G, the colour complex formed a solid mass at the water-chloroform interface. This could be overcome by gentle extraction with n-butanol instead of chloroform.

ION EXCHANGE—

In the ion-exchange methods employed, aminoethylcellulose in the form of Whatman's AE.50 floc or powder or AE.30 paper was used. This is a weakly basic anion-exchange material that absorbs acid dyestuffs from acid solutions (at a pH below 4 to 5). After the aminoethylcellulose had been thoroughly washed, the colour was stripped with dilute ammonia solution. Hence this material acted in a manner similar to that of wool, but required no heating. It was very useful for absorbing the colour from large volumes of solution, thus achieving concentration without the use of heat. The floc and powder were used in the form of short columns (say 5 cm long, 1 to 1·5 cm in diameter), the powder being mixed with about 3 parts of Kieselguhr to improve its porosity. Typical working instructions were those for boiled sweets—

> Dissolve about 20 g of sweets in cold water, and, if not already acid, acidify with acetic acid; pass the solution through a 1-inch column of Whatman's AE.50 floc. Wash the floc well with extremely dilute acetic acid, and then with water. Strip the colour from the floc with a little dilute ammonia solution (the strength required varies with the dye from about 1 to 10 per cent.*), collecting the coloured portions only. A few millilitres only should be required, but the ammonia can be removed by aspiration or during concentration under reduced pressure.

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* All concentrations of ammonia solution given in percentages refer to volumes of ammonia solution, sp.gr. 0·88, in 100 volumes of water.

Similar methods were also used for fondant, toffees, fish paste, and madeira cake. Whatman's AE.30 paper was used to purify the colour extract from toffees in the following manner after concentration under reduced pressure as described above—

> Take up the residue in a few millilitres of water, and make just acid to litmus with 1 per cent. hydrochloric acid. Pass the solution through a double disc of Whatman's AE.30 paper in a Gooch crucible under suction. Pass the filtrate repeatedly until all the dye is absorbed. Wash the filtrate with 20 ml of cold water, and strip it by passing a minimum volume of 10 per cent. ammonia solution through it.

CHROMATOGRAPHY—

Solutions of extracted dyes in isobutanol or n-butanol were sometimes purified by chromatography on an alumina column, *e.g.*, the solvent method described for canned peas can be continued—

> Pass the combined extracts through a ¾-inch bore polythene tube packed with dry alumina. Cut the column to isolate the coloured bands, and stir each fraction in a small beaker with 5 to 10 ml of 30 per cent. v/v ethanol. After the alumina has been allowed to settle, the supernatant liquors can be applied directly to chromatography paper.

APPLICABILITY OF METHODS—

All the methods were not carried out on all products containing the whole range of dyes. Alternative methods were employed only when the first method tried did not give an apparently complete recovery of the colour. For some dyes difficulty was experienced in obtaining a complete extraction into solvent, a complete take up on ion-exchange material or a complete removal from that material, but it should be emphasised that for a simple identification, not requiring complete recovery, any of these methods should be satisfactory.

With fresh sausages, methods involving the use of columns were unsatisfactory because the columns became blocked by colloidal proteins; this difficulty was not experienced with fish paste, presumably because much of the protein had been denatured by heat. Solvent methods were normally satisfactory for fresh sausages.

Ion-exchange methods were found satisfactory and very convenient with simple systems such as plain boiled sweets and fondants. With the exception of certain colours they were also found satisfactory with products, such as toffees and fish paste, containing cooked protein.

The colours that caused difficulties were Yellow 2G, Blue VRS, Black PN and Erythrosine. Erythrosine was extracted satisfactorily from an acid solution by ether. Blue VRS was found to be much more readily extracted into n-butanol than into isobutanol, and its partition was found to be strongly affected by pH, too acid a solution giving a poor extraction. Table I shows the results of solvent and cetylpyridinium methods as applied to these difficult dyes in toffees and fish paste.

TABLE I

SOLVENT AND CETYLPYRIDINIUM COMPLEX METHODS APPLIED TO TOFFEES AND FISH PASTE

	Toffees		Fish paste	
Colour	Solvents	Cetylpyridinium complex	Solvents	Cetylpyridinium complex
Blue VRS	Unsatisfactory	Satisfactory	Satisfactory with n-butanol	Satisfactory
			Unsatisfactory with isobutanol	Satisfactory
Erythrosine ..	Not tried	Satisfactory	Unsatisfactory	Not tried
Black PN	Satisfactory	Satisfactory	Unsatisfactory	Unsatisfactory
Yellow 2G ..	Not tried	Satisfactory	Satisfactory	Not tried

Once an initial separation of the colour had been made, other methods, unsatisfactory for the initial separation, were usually found quite satisfactory for further purification, suggesting that the problem was probably one of interfering impurities such as soluble proteins, amino acids, etc.

TABLE 11

CHANGES OCCURRING DURING PROCESSING AND STORAGE

n.c.p.—no change on processing n.c.s.—no further change on storage C.I. numbers refer to the 1956 colour index

Colour	Original dye	Boiled sweets	Fondants	Toffees	Fish paste	Madeira cake	Fresh sausages
Tartrazine CI 19140	No subsidiaries	n.c.p.	—	n.c.p.	n.c.p. Traces of mauve and brown after 15 months' storage	n.c.p.	n.c.p.
Sunset Yellow F.C.F. CI 15985	3 subsidiaries 0·42%, 0·32%, 0·42%	n.c.p.	—	n.c.p.	n.c.p.	n.c.p.	n.c.p.
Amaranth CI 16185	2·2% of Fast Red E present	n.c.p.	—	New red produced. Fast Red E partly faded	Yellow- brown colour produced	n.c.p.	n.c.p.
Ponceau 4R CI 16255	Orange-red and yellow subsidiaries, total 0·9%	n.c.p.	—	Subsidiaries increased	Yellow subsidiary greatly increased. Pink produced. Yellow increased after 14 months' storage as intense as main colour	n.c.p.	n.c.p.
Carmoisine CI 14720	0·4% red subsidiary	Red and purple on processing	—	n.c.p.	Yellow and orange on processing. Additional yellow, orange, pink and brown after 14 months' storage	n.c.p.	n.c.p.
Red 10B CI 17200	Mauve, blue and pink subsidiaries, total 0·9%	Additional blue on processing	—	Mauve subsidiary intensified. Orange-brown formed. Some subsidiaries lost, orange produced after 9 months' storage	Mauve subsidiary intensified. Blue subsidiary lost. Orange-brown formed. n.c.s. 13 months	n.c.p.	Red formed. n.c.s. 13 months
Red 2G CI 18050	0·2% pink subsidiary	n.c.p. n.c.s. 12 months	—	n.c.p. Trace of pink after 9 months' storage	Original colour almost gone. Several yellows formed. Mauve and traces of pink after 13 months' storage	n.c.p.	n.c.p.

TABLE II (*contd.*)

Colour	Original dye	Boiled sweets	Fondants	Toffee	Fish paste	Madeira cake	Fresh sausages
Ponceau MX CI 16150	Red subsidiary	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	Yellow formed. Yellow lost after 9 months' storage	n.c.p.	Subsidiary lost
PonceauSX CI 1470	No subsidiaries	Trace of pink n.c.s. 8 months	Trace of 3 pinks n.c.s. 8 months	n.c.p. n.c.s. 8 months	n.c.p. n.c.s. 8 months	n.c.p.	n.c.p.
Red 6B CI 18055	No subsidiaries	n.c.p. n.c.s. 9 months	n.c.p. n.c.s. 9 months	Trace of red. n.c.s. 9 months	Brownish yellow formed. Main colour lost after 6 months' storage. Yellow, orange, and more brownish-yellow formed	Trace of red	All colour lost on processing
Orange G CI 16230	Yellow and orange subsidiaries	n.c.p. Subsidiaries lost after 9 months	Trace of new colour found. Subsidiaries lost after 9 months	Red and new orange found. Subsidiaries lost after 9 months	n.c.p. Subsidiaries lost and new faint orange formed after 9 months	n.c.p.	n.c.p.
Yellow 2G CI 18965	2 yellow subsidiaries	n.c.p. n.c.s. 6 months	Traces of new colour formed	—	Subsidiaries lost n.c.s. 8 months	Trace of red	n.c.p.
Erythrosine CI 45430	At least 6 subsidiaries	n.c.p. Some subsidiaries lost after 7 months	n.c.p. Some subsidiaries lost after 7 months	n.c.p.	n.c.p. Faint brown after 7 months	Red and orange. Possibly original subsidiaries intensified	Yellowish
Blue VRS CI 42045	4 subsidiaries Mauve, blue, blue-green	n.c.p. n.c.s. 6 months	Trace of mauve	—	Trace of yellow n.c.s. 7 months	Trace of red	n.c.p.
Indigo-Carmine CI 13015	4 blue subsidiaries	—	Faint yellow. Yellow lost after 7 months	n.c.p.	n.c.p. except almost complete fading	—	n.c.p. except almost complete fading
Green S CI 44090	2 green subsidiaries	n.c.p.	n.c.p.	n.c.p.	n.c.p.	Yellow	n.c.p.
Black PN CI 28440	2 red, 1 orange 1 grey-blue subsidiaries	Yellow	Orange and yellow formed. 1 red subsidiary lost	n.c.p.	Orange	—	n.c.p.

SEPARATION AND IDENTIFICATION OF INDIVIDUAL DYES

The final process in every investigation made use of paper chromatography. These methods are adequately described in the A.P.A. Handbook, but the following additional notes should prove helpful.

SOLVENTS FOR PAPER CHROMATOGRAPHY—

The following solvents were used most frequently—

n-Butanol - water - glacial acetic acid—(20 + 12 + 5) by volume. (Solvent No. 5 of the A.P.A. Handbook.)

Ethyl acetate - pyridine - water—(11 + 5 + 4) by volume.

Ethyl methyl ketone - acetone - water—(7 + 3 + 3) by volume. (B.S.I. method for determining subsidiaries in Amaranth,[3] Ponceau 4R[4] and Red 10B.[5])

Ethyl methyl ketone - acetone - water - ammonia solution, sp.gr. 0·88—(700 + 300 + 300 + 2) by volume. (B.S.I. method for determining subsidiaries in Tartrazine,[6] Sunset Yellow,[7] Carmoisine,[8] Red 2G[9] and Yellow 2G.[10])

Trisodium citrate dihydrate - water - ammonia solution, sp.gr. 0·88—Trisodium citrate dihydrate (2 g) in 95 ml of water and 5 ml of ammonia solution. (B.S.I. method for determining subsidiaries in Orange G[11] and Ponceau SX.[12])

The B.S.I. solvents were used when appropriate, as the work described here was designed partly to look for changes in proportions of subsidiaries. However, it was often found desirable to extend the time for development when a more complete picture of the subsidiaries present was required; by using several different solvent mixtures, subsidiaries were often separated that would not have been found in a single solvent.

GENERAL COMMENTS ON PAPER CHROMATOGRAPHY—

The following general observations, some of which have already been made in the A.P.A. Handbook, are emphasised.

(*i*) R_F value alone is not a safe criterion for identification, even when several different solvents are used. R_F values are affected by such factors as temperature, length of run, paper variations (thickness, texture, machine direction, batch-to-batch variations), presence of impurities and other less easily defined conditions.

(*ii*) The use of standard dyes as markers is essential, but even with this precaution, impurities extracted with the colours, etc., may cause differences between the R_F values of the markers and extracted dyes.

(*iii*) The only safe method is to superimpose standard dye and extracted dye. If no separation occurs in any solvent system the colours may be assumed to be identical. At the same time spots of standard dye and extracted dye should be run separately at similar concentrations. This is necessary because the standard dye itself may split into more than one component and give misleading results.

(*iv*) Certain colours will produce two or more spots of identical composition when run in some solvents if the concentration on the paper is too high (*e.g.*, Tartrazine in A.P.A. Solvent 5).

RESULTS

The effects of processing the various colours so far examined are shown in Table II. No storage tests were carried out on fresh sausages or madeira cake, because both these products have only a short shelf-life.

Uncoloured material was also processed to ensure that yellow and brown spots on the chromatograms did not originate entirely from ingredients other than the dyestuffs.

Fading of colours on processing or storage is not reported here as it is almost universal, and no attempt has been made to investigate colourless substances produced by breakdown of the dyes.

Certain other changes have been reported in the literature.

Dickinson and Raven[13] reported that Ponceau 4R was reduced to a yellow colour by hydrogen sulphide or sulphur compounds liberated during the processing and storage of certain foodstuffs, and that Erythrosine lost iodine to produce fluorescein when canned cherries coloured with Erythrosine were stored in unlacquered cans.[13,14]

Ruiz and Laroche[15] found that Black PN was reduced in some sugar confectionery to give an orange colour that was not a known food dye. They also found that during extraction (on wool) this dye was partly converted to another orange colour that was not a known food dye.

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The Isolation and Separation of Dyes from Foodstuffs by Column Chromatography

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A method is described for isolating from foodstuffs, and then separating by column chromatography, twelve synthetic dyes used in Israel. Two adsorbents were used: alumina, at different pH values, and silica gel. The eluents used were water, n-butanol and methanol.

IN a previous publication[1] a paper chromatographic method was described for separating and identifying a group of twelve synthetic water-soluble food dyes in use in Israel. The procedure described can be applied directly to commercial dyes. Dyes to be determined in food must be extracted and then purified before they can be identified, since the impurities associated with food often cause considerable difficulty in identifying the dyes, by either paper chromatography, spectrophotometry or any other means of identification.

Wool has been used for extracting coal-tar dyes from food-stuffs,[2,3,4] and by suitable modification of this method, basic dyes could be separated from acidic ones.[5,6] Helberg[3] suggested the use of solutions of different pH values for eluting the adsorbed dyes from the wool. This procedure involved the use of strong acids and heat, conditions liable to alter the chemical structure of the dyes. Other procedures for isolating and separating dyes include solvent extraction at different pH values[7] and column chromatography.[8,9] Kaolin, alumina and silica gel have been used as adsorbents in chromatography.[10,11,12,13,14] The ninth edition of the "Official Methods of Analysis" of The Association of Official Agricultural Chemists, describes a method for separating dyes by using a powdered-cellulose column and a series of sodium chloride solutions as eluents.[15] This technique, however, was not always found to give the desired separation.[16]

A procedure for extracting dyes from foods and separating them by column chromatography is described in this paper. By using silica gel and alumina adjusted to different pH values, and with water, butanol and methanol as solvents, the dyes tested could be separated. The adsorbents acted as partition, adsorption or ion-exchange columns, depending on the conditions used. The dyes were obtained chemically pure, and could subsequently be identified with ease.

METHOD

REAGENTS—

All reagents used were of analytical-reagent grade.

Hydrochloric acid, 2 N *and* N.

Ammonia solution, sp.gr. 0·88, *and* N.

Ammonia water—Dilute 1 ml of ammonia solution, sp.gr. 0·88, with 99 ml of water.

Methanol, redistilled.

Methanol, 90 *per cent. v/v.*

Ammoniacal methanol—Dilute 1 ml of ammonia solution, sp.gr. 0·88, with 99 ml of methanol.

n-Butanol, dry.

n-Butanol—Saturate with 2 N hydrochloric acid by shaking the two reagents together for a few minutes.

Chloroform, redistilled.

Acetone, redistilled.

Sodium hydroxide solution, 5 N.

Sodium acetate solutions, M, 0·5 M *and* 0·1 M *aqueous.*

DYES—

Ponceau 4R
Red 6B
Tartrazine
Green S
Carmoisine
Red FB
Naphthol Yellow S
Blue VRS
Amaranth
Ponceau SX
Sunset Yellow FCF
Indigotine

ADSORBENTS—

Silica gel—M.F.C. Materials for Chromatography, Hopkin & Williams Ltd.

Basic alumina—B.D.H. Chromatography grade.

Acid alumina—Place about 500 g of the basic alumina in a 5-litre Erlenmeyer flask, and cover with N hydrochloric acid to about 10 cm above the surface of the adsorbent. Shake the Erlenmeyer flask occasionally for about 1 hour, and set aside overnight. Decant the acid. Cover the alumina with a further portion of N hydrochloric acid. Shake again occasionally for an hour, and decant the acid. Wash the alumina thoroughly several times with distilled water until the pH of the washings reaches 3 to 3·5. Filter off the alumina on a Buchner funnel, transfer to a flat tray, and set aside to dry at room temperature.

Neutral alumina—Place about 100 g of the acid alumina in a 1-litre Erlenmeyer flask, and cover with N ammonia solution to about 3 cm above the surface of the adsorbent. Shake the Erlenmeyer flask for about an hour, set aside overnight, and decant. Cover the alumina with a further portion of N ammonia solution, and shake again for an hour. Decant and wash the alumina with warm distilled water until free of chloride, and then filter it off on a Buchner funnel. Dry the alumina first at 110° C, and then activate it at 300° C for 3 hours.

PREPARATION OF COLUMNS—

Columns of 20-cm length and 2-cm diameter were used. Place a piece of cotton- or glass-wool at the bottom of the column. Fill loosely with the appropriate adsorbent to a height of about 5 cm, and cover with another piece of wood. Wet the adsorbent with the required solvent by applying slight pressure.

PROCEDURE—

Extraction of the dyes from foodstuffs—The procedure for extracting dyes added to food stuffs differs according to the type of food, and for this purpose the foodstuffs may be divided into two main categories.

I. Foodstuffs soluble in, or miscible with water:
  (*a*) Soft and alcoholic drinks, sweets, jellies, etc.
  (*b*) Milk.

II. Foodstuffs sparingly soluble, or insoluble, in water:
  (*a*) Non-fatty foodstuffs, like cereals or jams.
  (*b*) Foodstuffs containing fat, *e.g.*, butter, sausage or cheese, and non-fatty food containing natural pigments, *e.g.*, paprika.

I (*a*) Dissolve the foodstuff in a suitable amount of water at room temperature, or on a heated water-bath. Adsorb the solution on an acid-alumina column, and wash the column with 100 to 200 ml of warm water (between 50° and 70° C); liquid preparations may be adsorbed directly on the acid-alumina column. Elute the adsorbed dyes with ammonia water, and evaporate the eluate to dryness on a water-bath.

I (*b*) Add three volumes of acetone to one volume of milk, shake and allow the precipitate to settle. Collect the precipitate on filter-paper, and wash with small amounts of acetone until the dye is completely extracted. Apply the acetone solution onto an acid-alumina column and elute with warm water and then ammonia water as described under I (*a*).

II (*a*) Crush or cut the foodstuffs into small pieces and grind them in a mortar with 2N hydrochloric acid to a homogeneous paste. Extract the paste with 10-ml portions of butanol saturated with 2 N hydrochloric acid, and decant the liquid into a separating funnel; three or four portions of acid - butanol mixture will extract most of the dyes. Only Green S and Blue VRS will remain mainly in the paste and pass only partly into the butanol. Extract these two dyes from butanol with ten to twelve 5-ml portions of 2 N hydrochloric acid.

Adsorb the butanol solution containing all the dyes (except Green S and Blue VRS) on an acid-alumina column; wash and elute as described under I (*a*).

Add the acid extract containing Green S and Blue VRS to the original paste. Triturate the paste well, and filter. Wash the precipitate with 2 N hydrochloric acid until all traces of the dyes are removed from the paste. Adjust the pH of the acidic solution to approximately 1 with 5 N sodium hydroxide, and adsorb on to a silica-gel column. Elute the dyes from the silica-gel column with ammoniacal methanol, and *immediately* evaporate to dryness on a water-bath.

II (*b*) Crush or cut the foodstuffs into small pieces, and transfer them to an Erlenmeyer flask. Extract the mass in the Erlenmeyer flask with 20-ml portions of chloroform until the latter is colourless, while the flask is being shaken mechanically. Add 20 ml of N hydrochloric acid to the food mass, and extract again in the Erlenmeyer flask with 20-ml portions of chloroform. Remove the chloroform each time by decanting into a separating funnel. Return the hydrochloric acid solution each time to the food mass in the Erlenmeyer flask, and continue the extraction until the chloroform layer is colourless. Discard the interfering natural pigments and any unlisted dyes present that are soluble in chloroform. Extract the acid solution and the food mass with 10-ml portions of butanol until all the dyes have been extracted from the food. If Green S and Blue VRS dyes are suspected to be present in the sample tested, extract the combined butanol solution with ten to twelve 5-ml portions of 2 N hydrochloric acid, and add the acid extracts to the original acid solution. Adjust the pH of the acidic solution to approximately 1 with 5 N sodium hydroxide, and proceed as outlined under II (*a*). If the dyes Green S and Blue VRS are not expected to be present, combine the butanol extracts and adsorb on an acid-alumina coloumn, wash, and elute as described under I (*a*).

CHROMATOGRAPHIC SEPARATION OF THE DYES

SEPARATION OF THE DYES GREEN S AND BLUE VRS—

Dissolve the dyes obtained by one of the procedures outlined above (not more than 1 mg of each dye should be present in the sample) in 10 to 20 ml of distilled water, and pour the solution onto a silica-gel column. All the dyes except Green S and Blue VRS move quickly through the column. Wash the column with 5-ml portions of distilled water. Blue VRS remains strongly adsorbed at the top of the column, while Green S moves slowly during the washing: continue until this dye reaches to about 0·5 cm from the bottom of the column. To separate all the dyes from Green S and Blue VRS, 20 to 30 ml of water are usually sufficient. However, should separation not be complete, a second silica-gel column may be inserted below the first. (This step may be omitted when Green S and Blue VRS have been extracted and separated from the food by procedure II (*a*) or II (*b*).)

The dyes Green S and Blue VRS are eluted from the silica gel with ammoniacal methanol, and the solution is *immediately* evaporated to dryness on a water-bath. To separate dyes Green S and Blue VRS from each other, dissolve the residue in aqueous butanol (1 part water *plus* 10 parts butanol), and adsorb the solution on a basic-alumina column. Wash the column with 10 ml of aqueous butanol. Blue VRS is eluted first, followed by Green S. Adsorb each dye solution separately on an acid-alumina column, and elute each with ammonia water.

The dyes eluted earlier from the silica-gel column with water (all 12 dyes except Green S and Blue VRS) are adsorbed on an acid-alumina column and eluted with ammonia water. Evaporate the solution to dryness on a water-bath, and dissolve the residue in 1·5 ml of water and then add 10 ml of butanol. Adsorb the solution on a basic-alumina column, and wash the column with butanol saturated with water. The dyes on the column separate into two distinct bands, an upper one, Fraction A (containing Ponceau 4R, Amaranth, Red 6B, Tartrazine, and Indigotine), and a lower one, Fraction B (containing Carmoisine, Red FB, Ponceau SX, Naphthol Yellow S, and Sunset Yellow FCF). Continue eluting the column with aqueous butanol until the lower Fraction B is completely eluted.

SEPARATION OF THE DYES IN FRACTION A—

Wash the column containing the remaining Fraction A with three 5-ml portions of methanol to remove the butanol, and then continue washing with 90 per cent. methanol. Indigotide, and then Red 6B are now eluted; each dye is adsorbed on a separate acid-alumina column. Remove the remaining dyes, Ponceau 4R, Amaranth and Tartrazine, from the first column, by eluting with 10 to 15 ml of ammonia water. Evaporate to dryness. Dissolve the residue in water, and adsorb on an acid-alumina column. Elute with 0·5 M sodium acetate solution. Three distinct bands appear on the column. The first to be eluted is Tartrazine, and then Ponceau 4R; Amaranth remains adsorbed on the column. Dilute the acetate solutions containing Tartrazine and Ponceau 4R with water, (one part of acetate solution *plus* 10 parts of water), and adsorb again on acid alumina. Wash the last three acid-alumina columns with distilled water to remove the excess of sodium acetate before elution of the dyes. Finally, elute all the five dyes, now separated on individual columns, with ammonia water.

SEPARATION OF THE DYES IN FRACTION B—

Adsorb the aqueous butanol solution containing the dyes of Fraction B on an acid-alumina column, and wash with water to remove the solvent.

Elute the dyes with ammonia water, and evaporate the solution to dryness on a water-bath. Dissolve the residue in 10 ml of methanol, and adsorb the solution on a basic-alumina column. Elute Sunset Yellow FCF by washing with 90 per cent. methanol, and elute the remaining dyes from the column with 0·1 M sodium acetate. Adsorb the acetate solution on neutral alumina.

Wash the column with about 40 ml of 0·1 M sodium acetate, which will elute Naphthol Yellow S. Continue the washing with four or five 10-ml portions of M sodium acetate, which elutes Carmoisine and Ponceau SX together leaving Red FB at the top of the column.

To separate Carmoisine from Ponceau SX, dilute the 1 M acetate solution with about 200 ml of distilled water, and adsorb again on acid alumina. Wash the column with water, and extract the dyes with ammonia water. Evaporate the solution to dryness on a water-bath. Dissolve the residue first in 1·5 ml of water, and then add 10 ml of butanol. Chromatographically separate the solution on a basic-alumina column, by using aqueous butanol (1·5 parts water *plus* 10 parts butanol) as solvent. Carmoisine separates from the column.

Adsorb the eluted dyes Carmoisine, Naphthol Yellow S and Sunset Yellow FCF on acid-alumina columns, wash with water, and elute with ammonia water. Wash the column containing Red FB with water, and then elute the dye with ammonia water. Remove Ponceau SX directly from its column with ammonia water without previous washing with water.

The isolated food colours can now be identified by paper chromatography.[1]

DISCUSSION OF METHOD

Most of the synthetic dyes used for colouring foods are azo-compounds, containing various acidic and basic groups of different strength. These functional groups, their type, position and number in the dye-molecule, permit separation of the dyes by column chromatography. The two adsorbents used (silica gel and alumina, in their various forms described above), together with the three eluents (water, butanol and methanol), provide a wide range of adsorption, partition and ion-exchange conditions.

Silica gel adsorbs only dyes with basic groups from aqueous solution.

Acid alumina strongly adsorbs dyes with acid groups, and permits removal of extraneous material.

It is possible to separate the dyes into groups or individual colours by using alumina of different pH values.

By the method described, about 100 μg of a dye may be recovered from up to 500 g of food. In starchy gelatinous food a minimum of about 500 μg of dye must be present in 100 g of a sample.

The dyes are separated and isolated without application of either heat or strong reagents. This will be advantageous when working with compounds liable to decompose.

The extraction of small concentrations of dyes from food required the use of large amounts of solvents. By passing diluted solutions of dyes through appropriate columns, the dyes were adsorbed and the solvents recovered. Thus a dye solution could be concentrated without prolonged heating.

The use of small columns allowed the separated dyes to be obtained on single columns. In this way it was possible to proceed with the isolation and identification of some dyes (including those which remain strongly adsorbed on the column) before the complete separation of all the dyes had been accomplished.

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The Separation and Determination of Chromium Sesquioxide in Chrome Ores and Chrome-bearing Refractories

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Dichromate in solutions obtained from chrome ores and refractories by fusion can be almost completely removed by using liquid ion-exchange "resins" under slightly acid sulphate conditions. The chromium can then be quantitatively recovered from the organic phase by stripping with potassium hydroxide solution. After reduction, the chromium sesquioxide can then be determined spectrophotometrically as the complex with ethylenediamine-tetra-acetic acid to an accuracy within about 0·2 per cent.; this is more than adequate for routine control analysis. The aqueous phase is available for the determination of other constituents.

THE analysis of chrome ores and chrome-bearing refractories has been the subject of considerable study in the past few years. The current methods for analysing these materials are tedious, and the results often show considerable discrepancies between laboratories. Consequently, a more rapid and accurate method is needed and also one more suitable for routine control testing, when some accuracy may be sacrificed for the sake of increased speed.

Bryant and Hardwick's method[1] has been tested by the Chemical Analysis Committee of the British Ceramic Research Association and has proved to be a satisfactory procedure for the direct determination of chromium sesquioxide in these materials. Also, the same Committee has evaluated and published, in a private communication to the industry, a method for the accurate and direct determination of total iron. There is still a need for a method whereby the same solution of the sample can be used for determining both chromium and the other constituents. This is important, as the decomposition of the material is difficult and time consuming, and, therefore, it is undesirable to develop a series of methods for various constituents each involving a separate decomposition.

One of the main difficulties presented by this type of material is that the large amount of chromium present complicates the determination of many of the other constituents, and it is therefore desirable to separate this element before proceeding with the rest of the analysis. This paper represents a first step in a research programme, in that it describes a method for the prior separation and determination of the chromium to an accuracy adequate for routine control analyses.

In the past, chromium has been separated in this class of materials by one of three methods: volatilisation as chromyl chloride; electrolysis with the use of a mercury cathode; or by solvent extraction with isobutyl methyl ketone. Each procedure has its disadvantages. Volatilisation as chromyl chloride involves the decomposition of the sample with perchloric acid, which is not invariably successful, and, in addition, complete removal of the chromium is rarely achieved. Separation via a mercury cathode simultaneously removes the iron and manganese, and also there is a danger that some of the silica may be precipitated. Further, the subsequent cleansing of the mercury may give rise to difficulties in some laboratories. Extraction with isobutyl methyl ketone is the most useful of these techniques, but is invariably incomplete owing to the reduction of some of the chromate by the organic phase in the presence of the necessary hydrochloric acid. Often the amount of the residual chromium is tolerable, but occasionally the amount can be high enough to introduce significant errors.

Recently, liquid ion-exchange "resins" have become available, and these appeared to offer the possibility of removing chromate from slightly acid solutions. Green's work[2] on the removal of iron, titanium and zirconium suggested that the removal of chromium would be almost specific. Stripping the chromate back from the "resin" could then be accomplished by using alkali.

LIQUID ION-EXCHANGE "RESINS"—

The "resins" used in this work were manufactured by the Rohm and Haas Company of America and were obtained from their agents in this country, Charles Lennig and Co. Ltd., 26–28 Bedford Row, London, W.C.1. They are Amberlite LA–1 and LA–2, liquid secondary amines insoluble in water and having a high molecular weight. The "resins" are soluble in a large range of organic solvents, and that in which they are generally supplied is stated to be toluene.

EXPERIMENTAL

Preliminary trials showed that when a slightly acid solution of potassium dichromate in sulphuric acid was shaken with an approximately equal volume of "resin" solution (4 per cent. in chloroform) almost all the dichromate ion was transferred to the organic phase. When this organic phase was shaken with N potassium hydroxide the chromate ion was stripped from the "resin" and returned to the aqueous phase.

The solutions used in subsequent experiments were made to simulate those that would be obtained after fusion of a 0·5-g sample in sodium carbonate and boric acid and dissolution of the melt in sulphuric acid. A blank solution slightly acidified with sulphuric acid and containing 7 g of sodium carbonate and 2·5 g of boric acid per aliquot was used.

The effects of acidity, type of "resin" and solvent were investigated. The strength of the "resin" solution was increased from that used by Green to 10 per cent. by volume in the chosen solvent.

EFFECT OF ACIDITY—

The pH of the blank solution used for these experiments was found to be 2·4. Separations with "resin" LA–1 were carried out by using in the first extraction 25 ml of the "resin" solution and with 0-, 10-, 20- and 30-ml additions of dilute sulphuric acid (1 + 9).

The competition of the sulphate ion reduced the efficiency of the extraction, some dichromate being left in the aqueous phase. Two further extractions, each with 10 ml of "resin" solution were therefore carried out resulting in a colourless aqueous phase. This phase was then washed twice with 25 ml of chloroform to remove traces of "resin." The aqueous phase was then tested for residual chromate and also for total chromium (see Table I).

TABLE I

EFFECT OF ACID CONCENTRATION ON THE EXTRACTION OF CHROMATE

Potassium dichromate equivalent to 125 mg of chromium sesquioxide was taken for each determination

Sulphuric acid (1 + 9) added, ml	Chromate found in aqueous phase, mg of Cr_2O_3	Total chromium found in aqueous phase, mg of Cr_2O_3
0	0·42	0·45
10	0·00	1·19
20	0·00	1·49
30	0·00	1·77

The results in Table I show that more complete removal of the chromate is achieved at higher concentrations of acid, but is offset by increasing reduction of the sexavalent chromium. This reduction may be due to impurities in the "resin," *e.g.*, ferrous iron, organic reducing agent, or both. Stripping the "resin" before use to remove the ferrous iron, if present, was ineffective, and attempts to remove the organic reducing agents by treating the "resin" with permanganate revealed that the permanganate was reduced almost indefinitely; hence it was concluded that the "resin" itself was being oxidised.

To minimise this reduction and still obtain a complete removal of chromate, no acid was added until after the first two extractions, then 10 ml of sulphuric acid (1 + 9) were added before the third extraction. This technique proved effective.

The pH of the solution had an initial value of 2·4; after the first extraction this value increased to pH 4·2 and after the second extraction to pH 4·6. After addition of the sulphuric acid the pH dropped to about 1·3 and thereafter increased only slightly. This would seem to indicate a definite loss of sulphuric acid into the "resin."

CHOICE OF "RESIN"—

Amberlite LA-1 and LA-2 "resins" are similar in type, but, of the two, LA-2 is the more basic, so that, provided the sulphate was not preferentially extracted, it could be expected to be the more effective.

Separations with each "resin" were carried out as indicated above. Again, total chromium and residual chromate were determined in the aqueous phases. In both instances the chromate content was negligible and the total chromium sesquioxide was 0·060 mg with LA-1 and 0·045 mg with LA-2. The latter figure is equivalent to 0·009 per cent. of chromium sesquioxide if a 0·5-g sample of material had been taken. LA-2 was chosen for the method, as it also showed less tendency to emulsify.

CHOICE OF SOLVENT—

Chloroform had been used exclusively up to this stage of the work, and as a slight reduction still occurred it was thought possible that a change of solvent might be beneficial. "Resin" having a concentration of 10 per cent. by volume was used throughout.

As the "resin" is already dissolved in toluene this was the obvious first choice. Total residual chromium sesquioxide with LA-2 was 0·015 mg. This was a considerable improvement over the results obtained with chloroform, but the use of toluene, which is lighter than the aqueous phase, introduces manipulative complications. If it had proved possible to determine the chromium sufficiently accurately for a referee method, toluene would have been the solvent chosen, but, as the final method for chromium is of routine accuracy, chloroform appears to be at least adequate.

Carbon tetrachloride was next tried, but was even less effective than chloroform, the residual chromium sesquioxide being 0·11 mg.

Mixtures of carbon tetrachloride or chloroform with toluene (so as to obtain a heavier organic phase) were not so effective, probably because separation of the phases was much slower.

POSSIBLE EXTRACTION OF OTHER CONSTITUENTS—

Silica—It was thought that silica might be partially extracted under these conditions. With the equivalent of 4·95 per cent. of silica present before the extraction, recoveries were 4·99 per cent. with Amberlite LA-1 being used and 4·90 per cent. with LA-2. These errors are probably not greater than the experimental error of the spectrophotometric method of determination.

Ferric oxide—Under the conditions of acidity used here, Green found that extraction of iron took place, but the results of experiments showed that when a blank fusion solution (see "Experimental" p. 878) was present no loss of iron occurred. Thus it may be that it is the concentration of the sulphate or competing ion that is the important factor.

Titania—No extraction of titania was observed. However, Green found that it was extracted in the presence of hydrogen peroxide, and, as this could provide the basis for a subsequent separation and determination, attempts were made to extract titanium from the solutions by this procedure. In all instances recoveries were found to be only about 50 per cent. of the amount present, so that again competition from the sulphate appears to restrict extraction.

Manganese oxide—No extraction of manganese oxide appears to occur, whether this is added in the manganous state or as permanganate.

STRIPPING THE "RESIN" PHASE—

A single extraction with N potassium hydroxide appeared to strip the "resin" completely, but a second extraction was made with the same reagent to ensure that no significant amount of chromium was left in the slightly cloudy emulsion in the organic phase.

DETERMINATION OF THE CHROMIUM SESQUIOXIDE—

Several methods for the determination of chromium were examined. Most of the accurate methods are based on its determination as chromate, but even by using such a technique as Bryant and Hardwick's, which involves an auxiliary oxidation, some chromate always appears to be reduced by the slight traces of "resin," and results were always low. Other methods for determining the chromic ion were attempted, including gravimetric techniques with 8-hydroxyquinoline and benzoic acid, but low results were recorded.

A volumetric method based on the formation of a complex with ethylenediaminetetra-acetic acid (EDTA) and back titration with zinc and with dithizone as indicator gave surprisingly sharp end-points in the strong purple solution, particularly if this is screened with Naphthol Green B to a dark blue with a slight tinge of green. But it became clear from the consistently low results that the amount of EDTA required to ensure complete reaction would mean excessively large back-titrations.

The presence of large amounts of sulphate ion rules out precipitation as barium or lead chromate.

Přibil and Klubalova's spectrophotometric method, as described by Welcher,[3] proved to be successful. The chromate is reduced to the chromic ion with sodium sulphite and the chromium complexed with a large excess of EDTA at a pH between 2 and 4. The violet colour of the complex is measured at 550 mμ.

Solutions containing various amounts of chromate, and a portion of the blank fusion solution were extracted into the "resin" and the combined organic phase stripped with potassium hydroxide. The chromium^{III} recovered from the acidified aqueous solutions was then determined spectrophotometrically. The results, expressed as percentage of chromium sesquioxide equivalent to the chromium^{III}, were—

Amount added ..	10	10	10	25	25	40	40	50	50	50
Amount recovered ..	10·0	10·0	9·8	24·9	24·9	40·1	39·9	50·0	50·0	49·8

It will be seen that the results have a slight negative bias, but the technique of calibrating the instrument on solutions of potassium dichromate would appear to produce answers accurate enough for routine control. However, Přibil states that the sulphate ion causes the results to be slightly low (an error of up to 1 per cent.), and as the solutions contain sulphate extracted into the "resin" it seems legitimate to calibrate the instrument on solutions that have been extracted into the "resin" in the presence of a blank solution. Table II shows the results on a range of samples, the results having been calibrated against pure solutions and solutions passed through the "resin."

TABLE II

DETERMINATION OF CHROMIUM SESQUIOXIDE IN DIFFERENT MATERIALS CONTAINING CHROMIUM

The results are the mean of duplicate determinations

Material	Accepted value for Cr_2O_3 content, %	Cr_2O_3 content found after calibration against— pure solutions, %	solutions extracted into "resin," %
Mag-chrome brick, A	11·90	11·8	11·9
	11·26	11·1	11·3
Chrome-mag brick, B	23·79	23·6	23·8
	29·54	29·2	29·5
Turkish chrome-ore	39·44	39·0	39·2
Philippine chrome-ore	34·04	33·9	34·2
Grecian chrome-ore (B.C.S. 308) ..	41·50	41·1	41·3

An accuracy to within 0·5 per cent. of chromium sesquioxide is required in routine control work. The results obtained by the proposed procedure, after calibration against blank fusion solutions extracted into the "resin," had a maximum error of ±0·2 per cent.; this is not significantly different from the error in the results obtained by Bryant and Hardwick's procedure. The method takes a little longer to complete, but has the considerable advantage that other determinations can be made on the aqueous phase without recourse to a further fusion. Work is continuing on this possibility, with particular reference to the determination of lime, which has been a stumbling block in the past.

METHOD

"RESIN" SOLUTION—

Prepare a (1 + 9) solution of "resin" by adding 50 ml of Amberlite LA-2 to 450 ml of chloroform and mixing.

DECOMPOSITION OF THE SAMPLE—

Accurately weigh 0·500 g of the finely ground sample, dried at 110° C, into a platinum crucible. Add 7 g of anhydrous sodium carbonate and 2·5 g of boric acid, and mix thoroughly.

Heat over a bunsen or Meker flame, slowly raising the temperature until the mixture begins to melt; keep at this temperature until melting is complete. Then raise the temperature slowly and steadily to the full heat of the flame (about 950° C). After about 5 minutes at this temperature, swirl the contents of the crucible every 2 minutes, making sure that the particles of the sample on the side of the crucible come into contact with hot molten flux.

If swirling is begun too early, it is difficult to detach unfused particles from the side of the crucible. Continue to heat and swirl at this temperature for 25 to 45 minutes depending on the nature of the sample.

Completeness of decomposition can be checked visually by the absence of unfused particles at the bottom of the melt. The type and position of the burner should be chosen so as to maintain oxidising conditions throughout.

Cool, place the crucible and lid in a 250-ml beaker containing 85 ml of water and 15 ml of diluted sulphuric acid (1 + 1), and warm until extraction is complete. Remove the crucible and lid from the beaker, washing them with the minimum volume of water. Cool the solution.

Add diluted ammonia solution (1 + 1) until the first formation of a permanent precipitate, and then re-dissolve in 1 or 2 drops of diluted sulphuric acid (1 + 1).

EXTRACTION OF CHROMATE—

Transfer the solution to a 500-ml separating funnel, A; the volume at this stage should not exceed 150 ml. Add 25 ml of dilute resin solution (1 + 9), shake for 1 minute, and allow to separate. Transfer the organic phase to a second separating funnel, B, washing the stem of funnel A with chloroform. Repeat the extraction with 10 ml of dilute resin solution, and again transfer the organic phase to funnel B.

Add 10 ml of dilute sulphuric acid (1 + 9) to the aqueous phase, and repeat the extraction with a further 10 ml of dilute "resin" solution, again transferring the organic phase to funnel B.

Remove the traces of "resin" from the aqueous phase by two extractions with 20-ml portions of chloroform, the chloroform layers being added to the combined "resin" solution in funnel B. Reserve the aqueous phase and washings from funnel A for any other determinations, if required.

To funnel B add 50 ml of potassium hydroxide solution (approximately N), and shake for 1 minute. Allow the layers to separate, and transfer the organic layer to the now empty funnel A. Add to this funnel a further 50 ml of the potassium hydroxide solution, and again extract. Discard the organic layer, and combine the two aqueous phases. Remove traces of "resin" by shaking twice with 10-ml portions of chloroform, discarding the chloroform layers.

Transfer the alkaline aqueous phase to a 450-ml beaker, add hydrochloric acid, sp.gr. 1·18, until the solution becomes acid, and then add 6 drops in excess.

Boil the solution to remove the chloroform and to reduce the volume to about 200 ml. Transfer the solution to a 250-ml calibrated flask, and dilute to 250 ml.

DETERMINATION OF CHROMIUM SESQUIOXIDE—

If the solution appears cloudy, filter 40 to 50 ml through a dry Whatman No. 42 filter-paper, discarding the first few millilitres.

Transfer a 20-ml portion of this solution to a 250-ml beaker, add 20 drops of hydrochloric acid, sp.gr. 1·18, and then 10 ml of a 5 per cent. solution of sodium sulphite, ***while stirring***. Dilute to about 50 ml with water, and boil for about 5 minutes. Cool to room temperature, add 10 ml of 5 per cent. EDTA solution, and then add ammonia solution, sp.gr. 0·88, until the first appearance of a permanent precipitate. Dissolve this precipitate by adding 20 drops of diluted acetic acid (1 + 1), and dilute to 200 ml. Boil for 10 to 15 minutes, cool, and dilute to 250 ml in a calibrated flask.

Measure the optical density of the solution against water at 550 mμ in 4-cm cells. Determine the chromium sesquioxide content of the solution by reference to a calibration graph prepared either from pure solutions of potassium dichromate or by passing dichromate solutions mixed with a blank fusion solution through the separation process.

We thank the Director of Research of the British Ceramic Research Association, for permission to publish this paper.

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The Determination of Residues of "Ruelene" in Milk

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A method is described for determining Ruelene insecticide (*O*-4-t-butyl-2-chlorophenyl methyl methylphosphoramidate) residues in milk. After initial extraction from the milk with solvent, the insecticide is separated from interfering materials by paper chromatography. The Ruelene is then determined from its phosphorus content. The method described has a sensitivity of 0·05 p.p.m., and recoveries of Ruelene added to milk are 74 per cent. at a level of 0·5 p.p.m. and 80 per cent. at a level of 1 p.p.m.

RUELENE is the trade name of the Dow Chemical Company for *O*-4-t-butyl-2-chlorophenyl methyl methylphosphoramidate. This is a systemic insecticide that gives good control of warble fly in cattle. Before its use in treating dairy cattle could be recommended, studies on the rate of appearance and concentration of residues of Ruelene in milk had to be carried out, and to this end a chemical method for determining the pesticide at residual levels in milk had to be devised. The *in vitro* stability and recovery of Ruelene from milk had been studied by Timmermann *et al.*,[1] who used radioactive Ruelene. These studies showed that Ruelene was stable in milk for at least 14 days at 12° C and that recoveries of 90 per cent. could be achieved by using methyl cyanide and chloroform to extract the residues from milk.

The method described here was therefore based on this preliminary extraction. Ruelene insecticide is fat-soluble, and it was hoped to employ the clean-up procedure, described by Laws and Webley[2] in their general method, before determining the pesticide from its phosphorus content. However, it was found that Ruelene is strongly adsorbed on to alumina and could not be eluted by light petroleum alone. The use of mixed eluting solvents tends to remove traces of other phosphorus-containing compounds from the column thus increasing the blank value. It was decided, therefore, to investigate the use of quantitative paper chromatography. This was found to be satisfactory, and it was possible to obtain good recovery of the insecticide from the paper. The Ruelene was determined as phosphorus after wet ashing the insecticide with a mixture of nitric and perchloric acids.[2,3]

METHOD

APPARATUS—

Spectrophotometer—Unicam SP500, or any similar suitable instrument.

All-glass chromatography tanks—For descending-solvent chromatography.

REAGENTS—

All reagents should be of analytical-reagent grade and low in phosphate.

Chloroform.

Methyl cyanide—Purify by percolating through a column of neutral alumina.

n-Hexane.

Solvent system for chromatography—In a separating funnel shake trimethylpentane, methanol and water in the ratio 5:4:1. Allow the phases to separate. The upper organic layer forms the mobile phase, the aqueous layer the stationary phase.

Perchloric acid, N.

Sulphuric acid, N.

Nitric acid, sp.gr. 1·42.

Hydrochloric acid, sp.gr. 1·18.

Ammonia solution, sp.gr. 0·88.

Ammonium molybdate solution—Dissolve 50 g of ammonium molybdate in 400 ml of 10 N sulphuric acid, and dilute to 1 litre with distilled water.

Stannous chloride solution, 40 per cent. w/v—Dissolve stannous chloride dihydrate in hydrochloric acid, sp.gr. 1·18.

Stannous chloride solution, dilute—Dilute 0·5 ml of the 40 per cent. w/v solution to 100 ml with absolute ethanol. Prepare immediately before use.

Ethanolic sulphuric acid—Mix 5 ml of concentrated sulphuric acid with 245 ml of absolute ethanol.

Isobutanol - benzene mixture, (1 + 1) *v/v.*

Ruelene, 95 per cent. pure—Standard solutions in acetone.

Potassium dihydrogen orthophosphate, dry—For primary standards.

PRELIMINARY EXTRACTION—

Measure out 100 ml of milk into a separating funnel. If recovery experiments are to be carried out, add the Ruelene as a solution in acetone at this stage. Then add 150 ml of methyl cyanide, and shake the mixture thoroughly; this precipitates the milk solids. (Precipitation and coagulation can be aided by holding the separating funnel under a hot tap for about a minute.) Filter the supernatant aqueous methyl cyanide through a wad of glass-wool into a second separating funnel, wash the precipitated milk solids with 150 ml of chloroform, and add the washings to the methyl cyanide extract. Shake the combined extracts, and allow the phases to separate. Transfer the lower chloroform - methyl cyanide layer to a dry 500-ml Erlenmeyer flask, and re-extract the aqueous layer with a mixture of 35 ml of methyl cyanide and 35 ml of chloroform. Combine the organic phases, and dry for 1 hour over anhydrous sodium sulphate. Filter the dried methyl cyanide - chloroform extract into a dry distillation flask, and remove the solvent in a current of air. Transfer the oily residue quantitatively to a 100-ml separating funnel with 25 ml of methyl cyanide. Extract the methyl cyanide solution three times with an equal volume of hexane to remove fats and phospholipids.

Transfer the methyl cyanide extract to a 100-ml round-bottomed flask that has a small well blown in the bottom. Remove the solvent on a steam-bath, evaporation being aided by a gentle current of air. Wash down the sides of the flask with a small amount of methyl cyanide, and evaporate the solvent. Dissolve the residue in the well of the flask in a small amount of acetone (0·1 to 0·2 ml). The extract is then ready for chromatography.

CHROMATOGRAPHY—

The solvent system used is trimethylpentane - methanol - water in the ratio 5:4:1. Use the aqueous layer as the stationary phase and the organic layer as the mobile phase. The separation is carried out on Whatman No. 1 filter-paper by descending-solvent chromatography. To ensure saturation of the atmosphere with the stationary phase, line the walls of the chromatography tank with sheets of filter-paper soaked in the stationary phase.

The method of application of the extract to the paper chromatogram can be described as a process of "wicking." Wash a strip of Whatman No. 17 filter-paper, 5 cm long and 1 cm wide, with acetone. Cut one end of this strip to a point, and by using a Pasteur pipette apply the milk extract, together with the acetone washings of the well of the flask, as a band about 2 cm behind the point. Then stand the strip, point uppermost, in a small tube and place the point of the strip in contact with the paper chromatogram, which must be supported in a horizontal plane. Mark the paper chromatogram into lanes with light pencil marks, and locate the tip of the strip on the starting line. Add a small amount of acetone to the tube, thus applying the Ruelene to the chromatogram by means of ascending chromatography or "wicking." Keep the area of the spot discrete by evaporation with a gentle current of air applied to the upper surface of the paper. Allow this process to continue for about 45 minutes, by which time any Ruelene present will have been transferred to the chromatogram.

Then transfer the chromatogram to the glass tank, and set aside overnight to equilibrate. Add the mobile phase, and allow the chromatogram to develop for 3 hours. Then remove the chromatogram from the tank, and set aside at room temperature to dry. Inspect the chromatogram under ultraviolet light; the position of Ruelene can be identified as a dark spot. This spot has an R_F value of approximately 0·6 under the conditions described in this paper. Run standard solutions of Ruelene as markers to aid location. Mark the position of the spot.

By using a template, cut an area 2 inches long by 1 inch wide from the chromatogram at the position of the spot for Ruelene. Cut a similar area from the marker spot to act as a standard; cut a similar area from in front of the marker spot to provide a paper blank.

Transfer the pieces of paper to separate 100-ml digestion flasks, and to the contents of each flask add 10 ml of glass-distilled water and then 5 ml of concentrated nitric acid. Then add 4 ml of N perchloric acid and 1 ml of concentrated hydrochloric acid, together with a glass bead. Digest the paper under reflux for 40 minutes, then drain the water from the condensers, and heat the contents of the flasks to brown fumes. Remove the condensers, and heat the contents of the flasks until white fumes of perchloric acid appear. Care must be taken at this stage when paper is being digested as the contents of the flasks may ignite; this can be avoided by adding a further 1 ml of N perchloric acid, together with 2 to 3 ml of water. Wash the walls of each condenser with about 5 ml of glass-distilled water, and add the washings to the flasks. Heat the solutions until white fumes appear.

Set the flasks aside to cool, and add 10 ml of water. Gently boil the contents of each flask under reflux for about 30 minutes; this ensures the hydrolysis of any pyrophosphate formed in the latter stages of oxidation. Set the flasks aside to cool, add 4 ml of ammonia solution, sp.gr. 0·88, and boil off the excess of ammonia.

Transfer the contents of each flask quantitatively to separate 100-ml separating funnels calibrated at 10 ml, and dilute to the mark with distilled water. Add 3 ml of ammonium molybdate solution to the contents of each separating funnel, and mix the solutions. Add 9 ml of the isobutanol - benzene mixture, and vigorously shake the separating funnels for exactly 30 seconds. Allow the phases to separate, and discard the aqueous layer. Wash the organic phase once with 5 ml of N sulphuric acid, and again discard the aqueous layer; remove as much of the aqueous phase as possible at this stage, and dry the stem of each separating funnel with a strip of filter-paper. Then transfer the organic layer to a stoppered 10-ml graduated cylinder, rinse the separating funnel with a small amount of ethanolic sulphuric acid, and add the rinsings to the cylinder. Make the contents of each cylinder up to 9·6 ml, and add 0·4 ml of ethanolic stannous chloride solution. Mix each solution thoroughly, and then measure its optical density at 730 mμ in 1-cm cells against a blank solution prepared by using glass-distilled water in place of the test solution. Prepare a standard curve from known amounts of standard orthophosphate solution.

RESULTS

The recovery, by the proposed procedure, of Ruelene standards run as marker spots on the paper chromatograms is shown in Table I. These results have all been corrected for the paper blank value.

TABLE I

RECOVERY OF RUELENE STANDARDS

Ruelene present, μg	Ruelene found, μg	Recovery, %
50	45·0	90
111	121	109
50	46·5	93
53·5	48·0	90
50	51·0	102
55·5	54·5	98

The mean paper blank value (16 determinations) was found to be equivalent to 5·0 μg of Ruelene, with a range of 3·5 to 9·2 μg.

Samples (100 ml) of milk from untreated cows were analysed by the method described, and the results for the milk blank value corrected for paper blank value, were—

Ruelene equivalent, μg	4·0	8·0	4·2	2·7	1·7	5·7
Ruelene equivalent, p.p.m.	0·040	0·080	0·040	0·027	0·017	0·057

Recovery experiments were carried out on milk to which Ruelene had been added. The results of these experiments are shown in Table II, and have been corrected for both paper and milk blank values.

TABLE II

RECOVERY OF ADDED RUELENE

Ruelene added, µg	Ruelene found, µg	Recovery, %
100	80·2	80
111	88·0	79
55·5	41·5	75
55·5	40·5	73

These results show that residues of Ruelene in milk can be determined satisfactorily by this method. The mean milk blank value is equivalent to 0·044 p.p.m., which means that it should be possible satisfactorily to determine low levels of residues of Ruelene in milk. Preliminary experiments have shown that the appearance of residues of Ruelene in milk, after treatment of the cows, is transient and only low concentrations appear.

We thank Mr. F. G. Brown of Dow Agrochemicals Limited for supplying a pure sample of Ruelene and also Professor A. N. Worden and Mr. C. E. Waterhouse of the Huntingdon Research Centre for their interest and helpful discussions during the progress of this work.

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A Conductimetric Micro Method for the Simultaneous Determination of Carbon and Hydrogen in Organic Compounds

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A method is described in which the carbon dioxide and water vapour issuing from a modified Večeřa combustion train are absorbed in conductimetric cells, one containing potassium hydroxide and the other sulphuric acid; this permits carbon and hydrogen in organic compounds to be determined in 15 to 20 minutes. A similar method can be applied with a Belcher and Ingram train if nitrogen is absent.

Suggestions are made for a possible conductimetric determination of carbon, hydrogen and nitrogen.

In two previous papers[1,2] conductimetric micro methods for determining separately carbon and hydrogen in organic compounds have been described. In the second[2] of these papers it was briefly stated that the two conductimetric cells could be placed in series and the two determinations carried out simultaneously. It was further stated that future work would be aimed at substantially reducing the time required for such analysis. This paper describes the manner in which this aim has been fulfilled.

It was decided to work with sample weights of 1 and 5 mg, the latter weight being considered preferable for routine work, and to concentrate initially on applying the conductimetric finish to the rapid empty-tube combustion method described by Belcher and Ingram.[3]

DESCRIPTION OF APPARATUS

For work on 1-mg samples the two conductimetric cells were unchanged from the previous work, with the exception of the use of a skirted joint (see Fig. 1) in the hydrogen cell to reduce dead space. The two cells were joined by a small tube containing M.A.R. anhydrone (14 to 22 mesh), the connections being made with Neoprene tubing.

For work on 5-mg samples larger conductimetric cells were constructed, of similar design, but holding approximately five times the volume of electrolyte.

HYDROGEN CELL—

The electrodes consist of bright platinum wire (0·015 inch in diameter) of which about $\frac{1}{8}$ inch protrudes into the electrolyte. The plug carrying the thermistor is made from polytetrafluoroethylene. The over-all height of the cell is 40 cm; it has a capacity of 25 ml, and the length of the bubble path is 50 cm. At a flow rate of 25 ml per minute the contact time between the liquid and the bubbles of gas is about 10 seconds, and complete circulation of the electrolyte occurs every 90 seconds. (With a flow rate of 50 ml per minute the contact time is 5 seconds and the circulation time 60 seconds.) It is essential that the bore of the delivery tube is 1·5 mm to prevent undue condensation of water. The outlet from the cell is made through a skirted joint to keep dead space over the electrolyte to a minimum.

CARBON CELL—

Each electrode consists of 10 turns of bright platinum wire (0·015 inch in diameter) wound round a mandrel of $\frac{1}{16}$-inch diameter. The plug carrying the thermistor is made from either polythene or polytetrafluoroethylene. The over-all height of the cell is 55 cm; it has a capacity of 100 ml, and the length of the bubble path is 75 cm. At a flow rate of 25 ml per minute the contact time between the liquid and the bubbles of gas is about 15 seconds, and complete circulation of the electrolyte occurs every 60 seconds. (With a flow rate of 50 ml per minute the contact time is 10 seconds, and the circulation time is 45 seconds.)

The bore of the delivery tube is 2·0 to 2·5 mm with an outside diameter of 4 mm; this tube is sealed into a second tube that then fits into the larger sized glass coil (see Fig. 2), which is ground to fit the cell.

The two cells are mounted in a Perspex box and are joined by a small tube containing M.A.R. anhydrone (14 to 22 mesh), the connections being made with ball joints that are kept lightly greased with Apiezon grease.

The rest of the apparatus is similar to that used in the previous work, conductance being measured with a Guest, Keen & Nettleford conductance meter.

ELECTROLYTE—

The electrolytes for the cells were the same as those used in the previous work, namely, 99·83 per cent. sulphuric acid for the hydrogen cell[2] and 0·05 N potassium hydroxide for the carbon cell,[1] each cell being calibrated in the same manner as before.

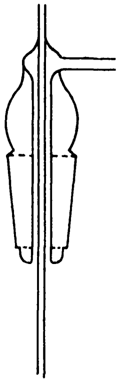


Fig. 1. Skirted joint used in hydrogen cell

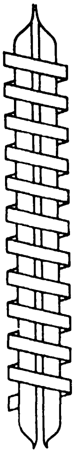


Fig. 2. Delivery tube of carbon cell

The mean factor from ten additions of dilute sulphuric acid to the hydrogen cell was 0·00480, expressed as mg of hydrogen per indicated ohm. The factor is operative up to the addition of 4·11 mg of hydrogen (36·7 mg of water), which permits the analysis of at least twelve 5-mg samples containing 5·0 per cent. of hydrogen.

The mean factor from ten additions of 0·05 N potassium carbonate to the carbon cell was 0·01696, expressed as mg of carbon per indicated ohm. The factor is operative up to the addition of 12·5 mg of carbon, which permits the analysis of three 5-mg samples containing 70 per cent. of carbon.

EXPERIMENTAL

During the work involving the use of the larger conductimetric cells it became apparent that, in this application, the Belcher and Ingram empty-tube combustion method was not suitable for the analysis of nitrogen-containing compounds; the nitrogen oxides on exit from the hot combustion chamber were not quantitatively retained by lead dioxide heated to 190° C, owing probably to the dissociation of nitrogen dioxide into nitric oxide and oxygen at temperatures in excess of 620° C, as suggested by Belcher and Ingram.[4]

$$2NO_2 \rightleftharpoons 2NO + O_2$$

These oxides of nitrogen were retained by the sulphuric acid in the hydrogen cell, resulting in high values for hydrogen. The method was satisfactory for all other types of compounds tested.

In view of this, Večeřa's combustion procedure,[5] in which cobalto-cobaltic oxide, Co_3O_4, at 620° C is used as catalyst, and an oxygen flow-rate of 20 to 25 ml per minute is maintained, was next examined as a more rapid method of pyrolysis for both 1- and 5-mg samples. This method proved to be completely satisfactory, the nitrogen oxides being quantitatively retained by lead dioxide heated to 190° C. The use of lead dioxide prolonged the recovery time of both water and carbon dioxide; without the reagent, carbon and hydrogen could be determined in

10 minutes by both the Vĕceřa and Belcher and Ingram methods on 1- or 5-mg samples. With the reagent in the Vĕceřa train, carbon and hydrogen could be determined in 15 minutes on a 1-mg sample and 20 minutes on a 5-mg sample, the limiting factor being the time taken for the combustion of the compound.

The filling used in the combustion tube for analysing 5-mg samples is shown in Fig. 3; when 1-mg samples are used, the length of the lead dioxide filling is reduced from 6 to 3 cm, the remaining space being filled with quartz chips.

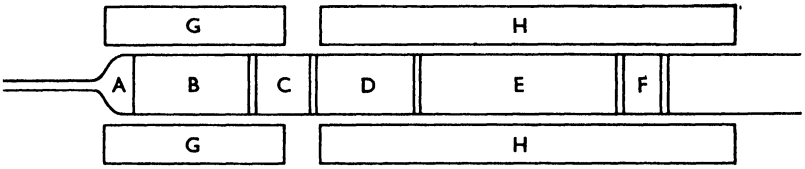
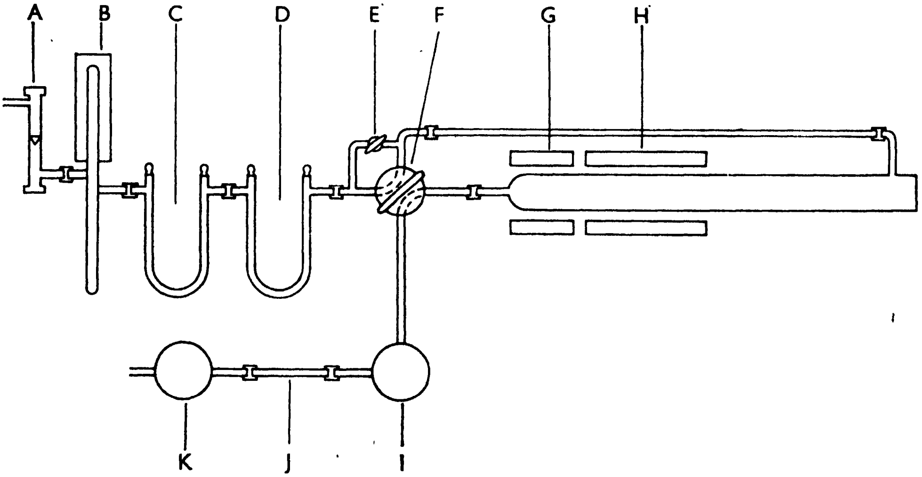


Fig. 3. Combustion tube fillings: A, quartz-wool plug; B, lead dioxide, 22 to 52 mesh (6 cm); C, quartz chips (2·5 cm); silver gauze (5·0 cm); E, cobalt oxide, 14 to 22 mesh (10·0 cm); F, silver gauze (1·0 cm). G, heating mortar; H, main furnace

Method

Assemble the apparatus as shown diagrammatically in Fig. 4. Wash and dry the cells in the manner described previously.[1,2] With oxygen flowing through the combustion tube at 25 ml per minute fill, by means of pipettes, first the hydrogen cell and then the carbon cell with the appropriate volumes of electrolyte. A piston-operated pipette fitted with a tap offers the most convenient way of dispensing the sulphuric acid. Ensure that no electrolyte enters the delivery tubes and that no air bubbles are trapped in the electrodes. Turn the main tap, F, to the by-pass position, and allow the cells to stabilise. This stabilisation period, which is only necessary when first filling the cells, takes approximately 10 minutes and is indicated by a constant reading (or drift) on the conductance meter. With oxygen flowing through the by-pass admit the sample into the combustion tube, and replace the bung. Close the main tap F in the oxygen line (see Fig. 4), and open pressurising tap, E; then open tap F to the combustion tube, and close the pressurising tap E. This procedure is essential to prevent electrolyte entering the delivery tubes of the cells. Should electrolyte



A = Rotameter
B = Pre-heater at 650° C
C = U-tube (6 inch) of AnalaR sodium hydroxide pellets
D = U-tube (9 inch) of M.A.R. anhydrone
E = Pressurising tube
F = Main two-way tap
G = Heating mortar at 190°C
H = Main furnace at 620°C
I = Hydrogen cell
J = Anhydrone tube
K = Carbon cell

Fig. 4. Assembled apparatus

enter the delivery tubes, the cells must be emptied, dried, and re-filled before the start of any analysis. Pyrolyse the sample in the usual manner, allowing from 5 to 7 minutes to complete the oxidation; avoid violent combustion. Continue the passage of oxygen through the combustion tube until the conductance meter reading for both cells is constant or indicates the drift value. Turn tap F to the by-pass position, and remove the sample container from the combustion tube. (The passage of oxygen for several hours through the combustion tube should be avoided, as this leads to over-drying of the lead dioxide, which results in the first hydrogen value being low.)

RESULTS

The proposed method has been applied to various organic compounds. The results for benzoic acid, indicating the standard deviation, the bias and the standard deviation of bias, are shown in Table I. The figures obtained by the gravimetric Pregl procedure are also shown for purposes of comparison. When the results of Table I are being interpreted it should be remembered that the sensitivity, and hence the accuracy and reproducibility, of the conductimetric finish can be varied by altering the strength or the volume of the electrolyte or both. The proposed method represents a compromise between the need for extreme sensitivity and the desire to do several determinations without refilling the cells.

Results for the carbon and hydrogen content of some other compounds are shown in Tables II and III.

TABLE I

RESULTS FOR HYDROGEN AND CARBON OBTAINED ON BENZOIC ACID BY DIFFERENT METHODS

Ten determinations were made by each procedure

Weight of sample taken, mg	Method of combustion	Finish	Results for hydrogen			Results for carbon		
			Standard deviation	Bias	Standard deviation of bias	Standard deviation	Bias	Standard deviation of bias
1	Pregl*	G	0·176	0·08	0·056	0·980	0·67	0·312
1	Pregl†	C	0·072	0·00	0·023	0·310	0·02	0·106
1	Věceřa†	C	0·036	0·04	0·011	0·115	0·02	0·035
5	Pregl*	G	0·022	0·03	0·007	0·250	0·01	0·080
5	Pregl†	C	0·056	0·02	0·017	0·157	0·07	0·044
5	Věceřa†	C	0·033	0·02	0·010	0·061	0·02	0·019
5	Belcher and Ingram‡	C	0·036	0·03	0·012	0·149	0·02	0·049

G. Gravimetric. C. Conductimetric.

* Manganese dioxide used as absorber for nitrogen oxides.

† Lead dioxide heating-mortar used.

‡ No nitrogen absorber used.

TABLE II

CARBON AND HYDROGEN FOUND IN 1-mg SAMPLES OF VARIOUS COMPOUNDS

A Věceřa train with a lead dioxide heating mortar was used. The weights of sample taken were in the range 0·900 to 1·150 mg. The drift on the hydrogen cell was 8 ohms per hour increase, and the drift on the carbon cell was 3 ohms per hour increase. Three determinations were made on each sample

Compound	Hydrogen content		Carbon content	
	Mean found, %	Theoretical, %	Mean found, %	Theoretical, %
m-Dinitrobenzene	2·44	2·38	42·72	42·86
Triphenylphosphine	5·83	5·72	82·16	82·44
Sulphonal	7·08	7·02	36·77	36·82
Cyclohexanone-2,4-dinitrophenylhydrazone	5·02	5·07	51·75	51·86

CONCLUSIONS

The determination of carbon and hydrogen by a simultaneous conductimetric finish has been successfully accomplished by suitable adaptation of the Věceřa train and the Pregl heating mortar. The procedure is now in routine use in these laboratories and is easily carried out by relatively inexperienced staff. The number of determinations of carbon and hydrogen that can be performed has been increased to more than twice that determined by the gravimetric Belcher and Ingram procedure previously used.

Experience in the removal of nitrogen oxides has suggested a possible means of determining nitrogen conductimetrically, when complete conversion of nitrogen to its oxides can be accomplished, since these oxides are absorbed in 99·83 per cent. sulphuric acid; experiments are now being made to verify this. If this determination can be made conductimetrically we will then attempt to determine carbon, hydrogen and nitrogen simultaneously. One way of doing this is to absorb the carbon dioxide in potassium hydroxide and the nitrogen oxides in sulphuric acid, after first absorbing the water vapour in a desiccant; subsequent heating of the desiccant would release the water vapour, which could then be absorbed in 99·83 per cent. sulphuric acid. Alternatively, it may be possible to split the gas stream and absorb each constituent separately.

TABLE III

CARBON AND HYDROGEN FOUND IN 5-mg SAMPLES OF VARIOUS COMPOUNDS

A Věceřa train with a lead dioxide heating-mortar was used. The weights of sample taken were in the range 3·75 to 5·25 mg. The drift on the hydrogen cell was 2·0 ohms per hour increase, and the drift on the carbon cell was <1 ohm per hour (negligible over the test period)

		Hydrogen content		Carbon content	
Compound	Number of determinations	Mean found, %	Theoretical, %	Mean found, %	Theoretical, %
m-Dinitrobenzene	4	2·35	2·38	43·10	42·86
Phenylthiourea	3	5·17	5·25	55·08	55·20
Bromobenzoic acid	3	2·46	2·49	41·97	41·80
Sulphonal	3	7·04	7·02	36·88	36·82
Triphenylphosphine	3	5·72	5·72	82·25	82·44
n-Butanol	3	13·56	13·60	65·06	64·80
Cyclohexanone-2,4-dinitrophenylhydrazone	3	5·04	5·07	52·10	51·86

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SHORT PAPERS

The Determination of Silica in Rocks and Minerals by Isotope Dilution with Silicon-31

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PRECISE and accurate methods are necessary for determining silica in rocks and minerals, particularly for purposes of rock classification. The classical gravimetric method for determining silica is time-consuming and has been shown to be subject to several sources of error.[1] Rapid spectrophotometric methods based on measurement of the blue colour of the reduced molybdosilicate complex[2] are also subject to interferences from certain ions in solution, particularly arsenate and phosphate.[3]

In the past few years, radiometric methods of chemical analysis have become common, and both neutron-activation and isotope-dilution methods have been applied to rock analysis. We have developed an extremely rapid and simple method for determining silica, which is accurate, reasonably precise and is not subject to most of the interferences affecting the gravimetric method. This method has been used for the routine determination of silica in silicates at the Mineralogical - Geological Museum, and is suitable for use wherever reactor facilities are available.

EXPERIMENTAL

The principle of the method is described below. A small amount of 2·6-hour silicon-31 (as active silica) is added to the powdered rock sample (ratio of active silica to silica in the sample, approximately 1 to 30); the mixture is fused with sodium hydroxide at 800° C and the cooled melt dissolved in water. The solution is acidified with hydrochloric acid and evaporated until most of the silica has precipitated. Evaporation to dryness must be carefully avoided as this increases the impurity content of the final silica. The silicic acid is removed by spinning in a centrifuge, washed thoroughly and ignited at 1000° C for 2 hours. A weighed portion of the silica is mounted on a planchet and counted with an end-window Geiger - Müller counter connected to a scaler. Standards of the active silica are also counted. The silica content of the rock is calculated from the expression—

$$\text{Percentage of silica in sample} = \frac{\left[\dfrac{m \times r_s \times 20}{r} - a\right] 100}{w}$$

Where, w = weight of sample taken, m = weight of silica in sample, r = count rate of silica in sample at time t, r_s = count rate of standard at time t and a = amount of active silica added.

Difficulty was found in obtaining sufficiently pure silica for irradiation. Irradiated Johnson Matthey 'Specpure' silica exhibited gamma activity, due to sodium-24, in addition to some longer lived beta activity. After irradiation the silica is fused with sodium hydroxide, the melt dissolved in water and the solution scavenged for impurities by adding ferric chloride; the resulting ferric hydroxide is removed by centrifugation. It was found that most of the foreign beta activity could be removed in this manner. The interference due to sodium-24 was eliminated by plotting the decay curves for the standards and correcting for the residual sodium-24 activity. The scavenged solution was used to prepare standards and to "spike" the samples.

In preparing the silica for counting it was found that not more than 30 mg of silica could be used, otherwise self-absorption of the beta radiation occurs. It was established experimentally that self-absorption is negligible if not more than 20 mg of silica are used.

NUCLEAR AND IRRADIATION DATA

Thermal neutron flux—approximately 10^{12} neutrons per sq. cm per second.

Reaction— $^{30}\text{Si}(\text{n},\gamma)^{31}\text{Si} \xrightarrow[T_{\frac{1}{2}} = 2\cdot62\ \text{h}]{\beta^-\ (1\cdot48\ \text{MeV})} {}^{31}\text{P}$.

Cross section of reaction—0·110 barns.

METHOD

APPARATUS—

Thin end-window Geiger - Müller tube and scaler—Obtainable from the Nuclear Chicago Corporation.

Circular stainless-steel planchets—31 mm in diameter and having a 25-mm diameter inscribed circle.

REAGENTS—

Silica—"Specpure," obtainable from Johnson Matthey & Co. Ltd.
Sodium hydroxide pellets—AnalaR grade.
Ferric chloride solution, 10 *per cent. w/v, aqueous.*
Silver nitrate solution, 1 *per cent. w/v, aqueous.*
Hydrochloric acid, sp.gr. 1·18—AnalaR grade.
Dilute hydrochloric acid (1 + 9).

PROCEDURE—

Weigh out 0·5 g of "Specpure" silica (dried at 110° C) into a small polythene tube, and heat-seal the ends. Irradiate the capsule in a nuclear reactor with a thermal neutron flux of 10^{12} neutrons per sq. cm per second for a period of 2 days. Set the material aside for several hours after irradiation to allow any short-lived activity to decay. Open the capsule, and transfer the contents quantitatively to a nickel crucible. Add 5 g of sodium hydroxide pellets, and heat the covered crucible at 800° C for 8 minutes. Allow the crucible to cool, then place it in a 200-ml beaker, add 100 ml of water, and heat on a steam-bath until the contents of the crucible have dissolved. Remove the crucible from the beaker, wash it, add 10 drops of ferric chloride solution, and heat on a steam-bath to complete precipitation of ferric hydroxide. Allow the solution to cool, filter, wash the filter-paper, and make the solution up to 250 ml in a calibrated flask.

Prepare five standards for counting by the procedure described below. Transfer 0·250 ml of scavenged silica solution by pipette on to a 25-mm diameter circle of tissue paper placed on a 31-mm diameter stainless-steel planchet. Evaporate the solution to dryness under an infrared lamp.

Weigh out 0·5 g of the sample into a nickel crucible, and by pipette add 5 ml of scavenged silica solution. Place under an infrared lamp, and evaporate slowly to dryness. Add 5 g of sodium hydroxide pellets, and fuse at 800° C for 10 minutes. Cool, add 15 ml of water, and heat the crucible gently to dissolve the cake. Transfer the contents to a 500-ml nickel evaporating dish, and wash the crucible thoroughly with dilute hydrochloric acid. Add 20 ml of concentrated hydrochloric acid to the contents of the evaporating dish, and place the dish on a steam-bath until the solution has evaporated to approximately 10 ml. (Most of the silica will have now precipitated.) Transfer the precipitate to a 15-ml centrifuge tube, and spin it. Wash the precipitate with 10 per cent. hydrochloric acid and then with water until the filtrate gives no positive test for chloride with a 1 per cent. solution of silver nitrate. Transfer the precipitate to a 15-ml quartz crucible, and ignite for 2 hours at 1000° C. Cool, and grind the silica to a fine powder in an agate mortar. Weigh out accurately 15 to 20 mg of the silica on to a steel planchet, and with water make an even deposit in the inscribed area. Dry under an infrared lamp. Prepare 10 such samples for counting.

COUNTING—

Immediately after their preparation, count the standards (at least 10,000 counts), and recount at frequent intervals over a period of 12 hours. Plot a curve of $\log_{10}$ activity *versus* time. Apply a correction for the longer-lived activity (^{24}Na) in the original silica solution. This correction can be calculated from the deviation of the curve from linearity. The samples should be counted as soon as possible after preparation, and not less than 4000 counts should be recorded. Apply a correction for decay of silicon-31 during counting and for coincidence, if significant. Read the count rate of the standard, at the time of counting the sample, from the decay curve of the standard. Calculate the silica content of the sample from the expression given on p. 891.

RESULTS

Listed in Table I are results for silica in several standard reference samples together with accepted values. Relative deviations are also listed to indicate precision. It can be seen that good agreement is obtained for all samples except N.B.S. 70. No reason for this lack of agreement was found.

Precision is within 2 per cent., and this will be satisfactory for many geological purposes in view of the simplicity and rapidity of the method. Statistical evaluation of the results for G-1 show that no bias is found in the distribution of the values, as occurs in the results obtained by the normal gravimetric method.[1]

TABLE I

RESULTS FOR THE DETERMINATION OF SILICA IN STANDARD SAMPLES

Standard sample	Number of determinations	Mean silica content, %	Relative deviation	Accepted value for silica content, %
G-1	39	72·56	1·70	72·65*
W-1	22	52·77	1·50	52·64*
N.B.S. 70 ..	18	67·49	1·90	66·63†
N.B.S. 99 ..	11	68·53	—	68·66†
Sulphide ore ..	2	34·28	--	34·6‡

* Values given in the "Second Report on a Co-operative Investigation of the Composition of Two Silicate Rocks."[1]

† National Bureau of Standards Certificate value.

‡ Value given in the "Report of the Nonmetallic Standards Committee," Canadian Association of Applied Spectroscopy.

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Modification of the Scott Method for the Determination of Indole

BY E. McEVOY-BOWE

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SCOTT[1] has published a method whereby indole is determined colorimetrically with *p*-dimethylaminocinnamic aldehyde after extraction from aqueous solutions with light petroleum. This method was shown by Scott to be 2·5 times as sensitive as that of the reaction with *p*-dimethylaminobenzaldehyde, and the green colour formed was claimed to be stable for several hours. Under the conditions in Singapore (temperature 30° C; relative humidity 80 per cent.) it was found that, although this method resulted in a far more sensitive colour development than that obtained with the *p*-dimethylaminobenzaldehyde reagent, the colour was unstable and faded at the rate 0·7 per cent. per minute after the first 15 minutes of colour development. To overcome this difficulty the procedure described below was developed; it has the advantages that the colour is stable for at least 2 hours under our conditions, and is about 4 times more sensitive than Scott's method, giving a linear graph within the range 0·5 to 5 µg or 0·05 to 0·5 µg of indole per ml in the original aqueous solution.

REAGENTS—

p-*Dimethylaminocinnamic aldehyde reagent*—A 0·5 per cent. w/v solution in n-propanol.

Sulphuric acid reagent—8 per cent. v/v of analytical-reagent grade concentrated sulphuric acid in 95 per cent. ethanol.

Acetic acid—50 per cent. v/v of analytical-reagent grade glacial acetic acid in water.

PROCEDURE—

Extract the indole from 10 ml of aqueous solution with 5 ml of toluene by shaking for 1 minute. Remove 4 ml of the toluene extract, and add to it 1 ml of the *p*-dimethylaminocinnamic aldehyde reagent. Place the mixture in a water-bath for 10 minutes at 37° C, and then add 0·5 ml of the sulphuric acid reagent, and allow the reaction to proceed for 10 minutes. Add 4 ml of the acetic acid, immediately remove the tube from the water-bath, and cool it to room temperature in tap water. Extract the colour into the acetic acid at the same time by gentle shaking for 1 minute. Allow the acid and organic layers to separate. Withdraw the lower acetic acid layer when it has cleared, and measure the colour in 1-cm cells with a suitable spectrophotometer at 625 mμ against a reagent blank solution. If difficulty is experienced in obtaining a clear acetic acid layer, either spin the extract in a centrifuge or take an accurately measured volume of the extract and add 0·2 ml of ethanol.

DISCUSSION OF THE METHOD

The extraction of indole with toluene results in the removal of 98 per cent. of the indole originally present in the aqueous phase. This was determined by measuring the absorption of the aqueous phase at 275 mμ before and after extraction.[1] n-Propanol is used as solvent for the *p*-dimethylaminocinnamic aldehyde reagent to allow complete miscibility of the sulphuric acid reagent with toluene. Fig. 1 shows the rate of colour development at 37° C.

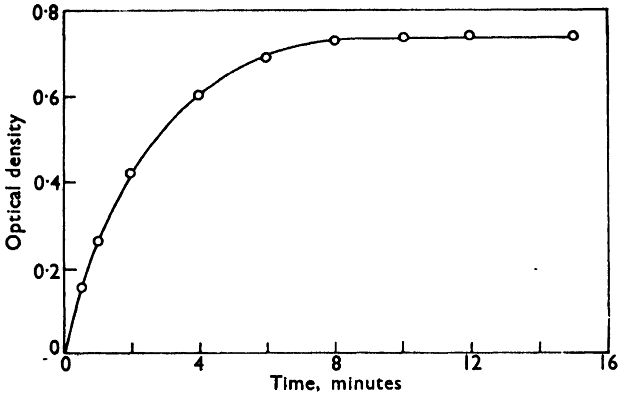

Fig. 1. Rate of development of the indole - *p*-dimethylaminocinnamic aldehyde colour at 37° C. In each test the colour development was stopped by extraction with 50 per cent. acetic acid; the optical density was then measured at 625 mμ

The use of acetic acid for the final extraction of the coloured complex has the advantage that the colour is stable in this solvent. Table I shows that the colour in toluene decreases slightly during 2 hours, whereas the colour in the acetic acid remains unchanged. Measurement of the coloured complex in toluene, before and after extraction with acetic acid, showed that 97 per cent. of the colour was extracted.

The standard colour development curve obtained after extraction of aqueous solutions containing known concentrations of indole was linear, passed through the origin and had a gradient of 0·134.

No colour was formed with any of the compounds named below: 3-methylindole, indole-3-ylpropionic acid, indol-3-ylaldehyde or indol-3-ylacetic acid. It appears, therefore, that non-substitution of the 3-position on indole is a specific requirement for the reaction, and that the method can be safely used for accurately determining indole in the presence of 3-methylindole.

TABLE I

STABILITY OF INDOLE COLOUR COMPLEX WITH *p*-DIMETHYLAMINOCINNAMIC ALDEHYDE

The reaction was carried out with 5 μg of indole in 4 ml of toluene, and the optical densities were measured at 625 mμ with a Unicam SP1400

	Optical density of complex in toluene	Optical density of complex extracted into acetic acid
10 minutes after removal from water-bath at 37° C	0·73	0·88
2 hours later	0·68	0·88

I thank Mr. Francis Tan for his invaluable technical assistance.

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Modification to the Two-column Chromatographic Apparatus used in the Determination of Vitamin A in Edible Fats

By RICHARD TURNER
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THE method proposed by Boldingh and Drost[1] for determining vitamin A in margarine has been adopted with slight modifications, as the official method[2] in the United Kingdom. Scott and Taylor[3] also used the modified method for determining vitamin A and carotene in butter.

A difficulty encountered in the two-column chromatographic procedure is in adjusting the rate of flow of the eluting liquid through the lower column. The apparatus is fitted with taps to control gas under pressure, which is applied to the columns to force the liquid through them. However, it is not easy to adjust the taps so as to maintain the necessary pressure differential. This becomes particularly inconvenient at the stage when the analyst is fully occupied collecting the eluate in tubes and testing their contents for the presence of vitamin A. By using a simple device, I have achieved good control over the rate of flow of the eluting liquid through the lower column.

Instead of connecting the upper and lower columns with a rubber tube, the opening for the tube in the upper column is plugged, and a 10-ml syringe (a hypodermic syringe is satisfactory) is connected to the lower column, as shown in Fig. 1.

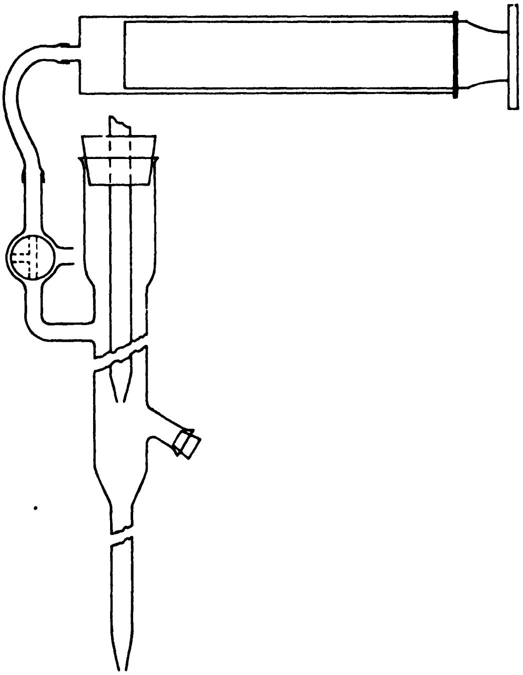

Fig. 1. Diagram showing how the syringe is connected to the lower column

Initially, the plunger of the syringe is set half way in the barrel. The level of the liquid in the lower column is raised or depressed by slight movement of the plunger. As a few seconds elapse before the new equilibrium is established, care should be taken to avoid over-adjustment.

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The Determination of *N*-Substituted Aromatic Thioureas

By PRAKASH CHANDRA GUPTA*

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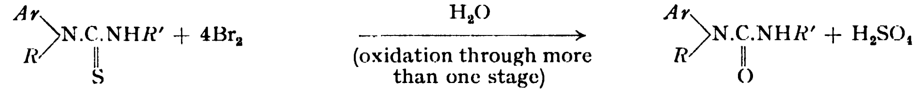
A SEARCH of the literature on the aromatic substituted thioureas revealed a lack of suitable methods for their determination. Callan and co-workers[1,2] reported that *sym*-diphenylthiourea could be titrated easily with bromine in glacial acetic acid at 25° to 30° C, 4 atoms of bromine being required per molecule of *sym*-diphenylthiourea. According to these workers substitution of bromine in the aromatic nuclei takes place. Wojahn reported that 1-naphthylthiourea is best determined argentimetrically[3] and that phenylthiourea could be determined with sodium hypoiodite.[4] Apart from these few attempts, no systematic work seems to have been carried out on the determination of aromatic thioureas (but compare other work[5,6,7,8]).

It has now been found that some aryl substituted thioureas can be determined titrimetrically with bromide - bromate mixture in an acid medium, and with iodine in a sodium hydrogen carbonate medium. The course of reaction with bromine can be represented by the general scheme shown below; the primary reaction is always removal of sulphur from the thiourea to give the corresponding urea.



$$\begin{matrix}Ar\\R\end{matrix}\!\!>\!\underset{\underset{S}{\|}}{\mathrm{N.C.NH}R'} + 4Br_2 \xrightarrow[\text{(oxidation through more than one stage)}]{H_2O} \begin{matrix}Ar\\R\end{matrix}\!\!>\!\underset{\underset{O}{\|}}{\mathrm{N.C.NH}R'} + H_2SO_4$$

where *Ar* = phenyl and
(*i*) *R* and *R'* = H,
(*ii*) *R* = H and *R'* = phenyl,
(*iii*) *R* = H and *R'* = methyl,
(*iv*) *R* = ethyl and *R'* = H.

The urea so produced undergoes bromination in the aromatic nucleus (or nuclei) to give the corresponding nuclear brominated products, with the exception of *N*,*N*-ethylphenylthiourea, which appears to behave like di-substituted anilines or those anilides substituted on the nitrogen attached to the aromatic nucleus.

Thus—

(*i*) $C_6H_5.NH.CS.NH_2 + 7Br_2 \xrightarrow{H_2O} C_6H_2.Br_3.NH.CO.NH_2 + H_2SO_4 + 11HBr.$

N-phenylthiourea 2,4,6-tribromophenylurea

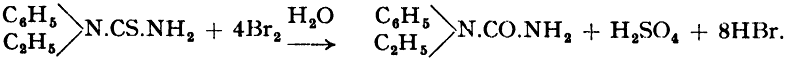
(*ii*) $C_6H_5.NH.CS.NH.C_6H_5 + 6Br_2 \xrightarrow{H_2O} Br.C_6H_4.NH.CO.NH.C_6H_4.Br + H_2SO_4 + 10HBr.$

N,*N'*-diphenylthiourea 4,4'-dibromodiphenylurea

(*iii*) $C_6H_5.NH.CS.NH.CH_3 + 7Br_2 \xrightarrow{H_2O} C_6H_2Br_3.NH.CO.NH.CH_3 + H_2SO_4 + 11HBr.$

N-methyl-*N'*-phenylthiourea *N*-methyl-*N'*-(2,4,6-tribromo)phenylurea.

(*iv*)



$$\begin{matrix}C_6H_5\\C_2H_5\end{matrix}\!\!>\!N.CS.NH_2 + 4Br_2 \xrightarrow{H_2O} \begin{matrix}C_6H_5\\C_2H_5\end{matrix}\!\!>\!N.CO.NH_2 + H_2SO_4 + 8HBr.$$

N,*N*-ethylphenylthiourea *N*,*N*-ethylphenylurea

According to the mechanisms outlined above, thioureas (*i*) to (*iv*) require 14, 12, 14 and 8 atoms of bromine, respectively, for complete reaction; this has been verified experimentally. The brominated products in equations (*i*), (*ii*) and (*iii*) have been isolated and identified.

EXPERIMENTAL

BROMIMETRIC DETERMINATION OF AROMATIC THIOUREAS—

In the direct titration of phenylthiourea solutions with bromine or bromide - bromate mixture in an acidic medium a sharp and reproducible end-point could not be obtained. Methyl red and phenol red, which were used as internal indicators, were readily decolorised before the true end-point was reached. Potentiometric titrations, which had been successful with thiourea and its alkyl homologues,[5,8] showed no improvement on the methods described above, as the changes in potential were rather slow and therefore untrustworthy. The procedure described below was found to give reproducible results.

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To a known volume (about 20 ml) of a standard solution of the thiourea in water or dilute acetic acid, acidified with 5 N hydrochloric acid, was added a known excess of 0·1 N bromide - bromate solution (prepared from analytical-reagent grade materials). The solution was set aside for 20 to 30 minutes, and potassium iodide was added. The iodine liberated (by the excess of bromine) was then titrated against standard sodium thiosulphate solution, in the usual manner. The number of atoms of bromine consumed per molecule of thiourea is shown in Table I.

The influence of a large excess of bromine on the determination was tested with a solution containing 0·3324 g of *N*,*N*′-methylphenylthiourea per litre. Portions of this solution from 2·0 to 30·0 ml were taken, and 10 ml of 5 N hydrochloric acid and then 10 ml of 0·1 N bromide - bromate were added to each; the excess of bromine was then titrated with 0·0944 N sodium thiosulphate. The results (atoms of bromine consumed per molecule of the thiourea) ranged from 17·7 for the 2-ml portion of original solution to the theoretical 14 for the 30-ml portion, indicating that results are high when too large an excess of bromine is present.

Experiments with larger amounts of the thiourea solutions and bromide - bromate mixture allowed the brominated products to be separated. Phenylthiourea gave the expected 2,4,6-tribromophenylurea (m.p. found, >270° C[10]; bromine found, 64·89 per cent.; calculated 64·3 per cent.). Extremely small amounts of dibromophenylurea were also formed (m.p. 201° C[10]) and were separated by fractional crystallisation. *sym*-Di-phenylthiourea gave the expected 4,4′-dibromodiphenylurea (m.p. found, >300° C[11,12]; bromine found, 45·0 per cent.; calculated, 43·19 per cent.). *N*-Methyl-*N*′-phenylthiourea gave the expected *N*-methyl-*N*′-tribomophenylurea (m.p. found, 222° C; bromine found, 59·7 per cent.; calculated, 61·97 per cent.—the slightly lower value found might be due to the presence of a lower brominated product). *N*,*N*-Ethylphenylthiourea gave a product melting at 74° C, free from bromine and containing 17 per cent. of nitrogen. The product could not be identified fully (m.p. of ethylphenylurea, 62° to 62·5° C[13,14]).

TABLE I

BROMIMETRIC DETERMINATION OF AROMATIC THIOUREAS

Thiourea	Solvent	Range of determination, g per litre	Atoms of bromine consumed per molecule of thiourea	
			Expected	Found
N-Phenyl-	Water	0·1522 to 1·5222	14	13·5
N,*N*′-Diphenyl-	Acetic acid	0·9132 to 9·1324	12	11·8
N,*N*′-Methylphenyl- ..	Water	0·3324	14	14·0*
	Acetic acid	3·3248 to 6·6496	12	12·0
N,*N*-Ethylphenyl-	Water	0·1803 to 0·3604	8	8·5

* Over-bromination causing high results can occur if the excess of bromine used is too large.

Iodimetric determination of aromatic thiourea

Direct titrations of phenylthiourea solutions with iodine were also unsuccessful, as the consumption of iodine per molecule of phenylthiourea was found in acidic medium to be between 1 and 2 atoms and in alkaline medium between 4 and 6 atoms. Phenylthiourea, *N*,*N*′-methylphenylthiourea and *N*,*N*-ethylphenylthiourea were successfully determined as described below.

Ten millilitres of the thiourea solution of known concentration were mixed with about 20 ml of M sodium hydrogen carbonate and a known excess of standard iodine solution. After about 20 minutes the excess of iodine was titrated with 0·1 N arsenous oxide. As expected, 1 mole of the thiourea was equivalent to 8 atoms of iodine; up to a concentration of about 1 g of the thiourea per litre, quantitative results were obtained.

Discussion of the method

Aromatic thioureas are not particularly soluble in water, which imposes a limit on the concentration range in which they can be determined in aqueous solution; within this range, however, they can be accurately determined with bromide - bromate solution in acid medium or with iodine in presence of sodium hydrogen carbonate.[5] The bromine consumption in the bromimetric determination was found to be slightly less than expected (except for *N*,*N*-ethylphenylthiourea), but in all determinations the results were reproducible within the limits of experimental error.

Only *N*,*N'*-methylphenylthiourea consumed more bromine than expected, the amount depending on the excess of bromine used.

The results of determinations with excess of iodine in presence of sodium hydrogen carbonate showed excellent agreement with the amount of sample taken, especially when dilute solutions were used. *sym*-Diphenylthiourea, which is almost insoluble in water, could not be determined iodimetrically. A search for non-aqueous media for the determination of aromatic thioureas is being undertaken.

I thank Professor S. S. Joshi and Dr. R. H. Sahasrabudhey for their keen interest and valuable suggestions and Dr. G. B. Singh, Head of the Department of Chemistry, for the facilities provided during this investigation.

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Received *January 15th*, 1963

Application of a Modified Stannous Chloride Reagent for determining Orthophosphate

By A. HENRIKSEN
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Two years ago Sletten and Bach[1] suggested that the stannous chloride used as reducing agent in the molybdenum blue method for colorimetric determination of orthophosphate should be dissolved in glycerol instead of in strong mineral acid. They claimed excellent stability for this reagent. Apparently, this modification has received little attention. We have used the modified reagent in our laboratory for more than a year with good results, and we believe it provides an important improvement in the molybdenum blue method.

In our routine determinations of phosphate in large amounts of seawater and, occasionally, in sewage effluents, we differentiate between orthophosphate and acid hydrolysable phosphates. To obtain this differentiation we chose the extraction method originally published by Martin and Doty[2] and later modification by the Association of American Soap and Glycerin Producers (AASGP).[3] A further advantage of the extraction step is elimination of interferences from most materials found in water.

We have modified two of the reagents used in the method described by the AASGP Committee. First, the stannous chloride reagent was modified by dissolving 0·125 g of $SnCl_2.2H_2O$ in 100 ml of analytical-reagent grade glycerol (phosphate-free). (One millilitre of this solution was used directly for reducing the yellow molybdophosphoric acid in the organic layer.) Second, the benzene - isobutanol mixture used for extraction was replaced by pure isobutanol, to avoid the use of benzene (a health hazard). The colour was measured in an E.E.L. photometer (Evans Electroselenium Ltd.) with a deep-red filter (609) and 4-cm cells being used. The visible spectrum of the colour formed shows two peaks, at 625 and 730 mμ.

Polarographic analysis of samples of the modified reagent stored for about 5 months at room temperature showed that only 16 per cent. of the stannous ions were oxidised to stannic ions, even though no precautions were taken to protect the solution from light and air.

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Received *April 18th*, 1963

Diffusion Bottles for the Determination of Fluorine

By R. J. HALL*

(*Soil Chemistry Department, National Agricultural Advisory Service, Brooklands Avenue, Cambridge*)

THE original bottle and cap described by Hall[1,2] for determining fluorine by diffusion as hydrofluoric acid is no longer available, but those at present supplied by Arnold Horwell Ltd., Cricklewood, London, also have a capacity of 20 ml, and can be used without modification. The sample and reagents are placed in the bottle, and the filter-paper (3 cm × 1·5 cm) is inserted into the neck so that 2 to 3 mm can be folded back over the rim. One drop of 0·1 N magnesium succinate is placed with a dropping pipette, having a fairly long capillary (7 or 8 cm), on that part of the paper that is free inside the bottle. The cap is quickly but carefully screwed on, and this alone holds the filter-paper in position. The cap is now sealed with hot wax as previously described. Recoveries from these bottles have been as quantitative and reproducible as with those formerly used.

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Received *July 23rd*, 1963

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* Present address: National Agricultural Advisory Service, Kenton, Newcastle-on-Tyne.

Book Reviews

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume I. ABHERENTS TO ALUMINUM. Edited by HERMAN F. MARK, JOHN J. MCKETTA, JUN., DONALD F. OTHMER and ANTHONY STANDEN. Second Edition. Pp. xx + 990. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price £16 18s.; price per volume for subscribers to the complete set of 18 volumes £13.

I think it was Oliver Wendell Holmes who, pondering on the remarkably well-informed conversation of a new acquaintance, suddenly realised that all the subjects discussed had started with the letter A; and that the first volume of a new encyclopedia had recently been published. He lost no time in contriving another meeting and leading the conversation to a subject lower down in the alphabetical scale—with the expected result! A conscientious reviewer of the first volume of the second edition of Kirk and Othmer might well find himself in the same position as Holmes' acquaintance, and be able to talk with full authority on subjects as widely separated but as alphabetically close as ablation, alcoholic beverages, aerosols, algal cultures and allyl compounds.

The first edition appeared in 15 volumes published between 1947 and 1956, with supplements in 1957 and 1960. The interval that has elapsed since the first edition of this encyclopedia has obviously necessitated much revision, expansion and addition, and it is claimed that the 1000 specialist authors participating in the present work have ensured the inclusion of the most recent advances in all fields of chemical technology. Emphasis is placed about equally on the scientific presentation of the physical and chemical properties of a substance and on its manufacture and uses. Fundamental concepts and unit operations of chemical engineering are dealt with in individual articles. It is intended to cover analytical methods in general articles, and there are to be numerous articles on general scientific subjects. There is an adequate bibliography for each major article.

It is difficult to assess the extent and efficiency with which all these claims have been implemented from the examination of a volume that considers only words up to AL. However from such tests as it has been possible to apply to the 35 major articles contained in the 990 pages of this volume, it would seem that the claims made as to scope, accuracy and documentation are fully justified. Moreover, if one considers the number of authors involved, the presentation is remarkably balanced and consistent, and in addition to this the layout is pleasing and easy to the eye. Indeed, for a scientific work the general standard of production may be described as almost lavish.

The analyst may be less satisfied. This volume contains no articles on analytical methods, and such references to chemical analysis as exist are relatively brief and superficial and occur in only a few of the articles. It is hoped that the subsequent volumes will restore the balance—the word "analysis" has still to come. At this stage however, I cannot offer any guide as to what help or benefit will accrue to my analyst colleagues.

Nor can I wholly subscribe to the international character claimed for this new edition. Compared with the first edition, which was intended to concentrate on United States technology, it certainly has broadened its frontiers. However, it is still predominantly American in approach and treatment, and a glance at the list of contributors and the bibliographies amply confirms this view.

One is tempted to compare this work with the nearest of its kind published in the United Kingdom, Thorpe's "Dictionary of Applied Chemistry," the last edition of which (11 volumes, each of about 600 pages) appeared spread over the years 1937 to 1954. Thorpe, when it was published, had an unassailable reputation for comprehensive treatment in a concise form combined with accuracy, and it has to be stated that in these respects it is equalled by Kirk and Othmer. However, it is important to appreciate the difference between a dictionary and encyclopedia. Thorpe combined much of the character of both; many words were defined, and selected words and subjects were discussed in articles of appropriate length. Kirk and Othmer is a true encyclopedia in that it has no specific definitions, and the articles are longer and mostly of a generalised nature, covering, as a rule, groups of substances rather than individual compounds. Thus one of the longest articles (89 pages) is headed "Alkali and Chlorine Industries," and it covers chlorine, sodium carbonate and sodium hydroxide. Under the heading "Acids," one finds only "acids carboxylic" and "acids dicarboxylic," with no reference to other acids, such as acids, aliphatic or acids, mineral. These no doubt, are discussed elsewhere. Industrial alcohol is now considered under "Ethanol" instead of under "alcohol" as in the first Edition; and in general a compound is discussed under its own name, under a group of substances or as a derivative under a parent compound. Even with the cross-references (which do not tell us where to find "alcohol"), this system may appear to make reference rather difficult when one has to handle all eighteen volumes of this size. No doubt a collective index will be available ultimately, as with the first Edition, but this of course must await the publication of the complete text.

This is obviously a very important work, and any opinion that has to be based only on the first of the 18 volumes must necessarily be guarded. This fraction however, represents a promising start and if, as appears likely, the analyst will not want to buy the series for its coverage of chemical analysis, he will certainly want to do so for the information it can give him on chemical technology as a whole. The word "want" is used advisedly because of the price consideration. Attention should however, be drawn to the substantial reduction of 23 per cent. offered by the set subscription price.

JULIUS GRANT

JOURNAL OF THE ASSOCIATION OF PUBLIC ANALYSTS. Volume 1, No. 1. First Quarter, 1963. Hon. Editor, DR. E. C. WOOD. Pp. 24. London: The Association of Public Analysts. Annual Subscription for 4 quarterly parts 30s.; single copies 7s. 6d.

A decade has passed since it was mutually agreed to separate certain functions of the Society of Public Analysts and Other Analytical Chemists by altering the name to the Society for Analytical Chemistry and by the formation of the Association of Public Analysts. Advantage has been gained by the changed emphasis of the Society and its Journal, and the Association has been able to concentrate on, and to expand, its own particular interests.

It appears, however, that for some time public analysts have felt the need for more practical interchange of ideas and for more information of what other public analysts are thinking and how they propose to tackle the various problems that concern their work. The circulation of confidential bulletins and of non-confidential monthly reports has not wholly met their needs. It was therefore decided to publish a quarterly journal, and the first number has now appeared. This contains a Foreword by the President of the Association, Special Announcements, Analytical and Technical Contributions and Items of Interest. The Special Announcements include the official view of the Association on Food Standards for Sausages and Sausage Meat, Luncheon Meat, Flavoured Milk, Marzipan, or Almond Paste, and Candied Peel or Cut Peel. Some of these standards have been arrived at by agreement with the Food Manufacturers Federation and will be of interest to all food chemists. The contributions are from public analysts only, but it is hoped that subscribers to the Journal and others interested will submit matters of mutual interest.

Public analysts were the founders of our Society and have always maintained close relations with chemists in all branches of the food industry. It is important that such liaison should be maintained, and the opportunity for food chemists to subscribe and contribute to this new journal should not be missed.

In his Foreword to this first number the President says "The Association has done much since its inception in 1953 to foster a spirit of co-operation, negotiation and mutual understanding between those responsible for making the laws relating to food and drugs, those responsible for their enforcement, and those who have to tread warily to avoid committing an offence." If, as it deserves, this Journal secures support from the wide field of food chemists, it will fill a very useful and important rôle in the public interest. J. R. NICHOLLS

PRACTICAL METHODS FOR THE MICROBIOLOGICAL ASSAY OF THE VITAMIN B-COMPLEX AND AMINO ACIDS. By E. C. BARTON-WRIGHT, D.Sc., F.R.I.C., M.I.Biol. Pp. 52. London: United Trade Press Ltd. 1963. Price 10s. 6d.

This inexpensive paper-covered book consists of a series of articles written for *Laboratory Practice* and is, in effect, a revised edition in cheaper format of the author's "Microbiological Assay of the Vitamin B-Complex and Amino Acids." The list of B-Vitamins appearing in the earlier book has been extended to include folinic acid and vitamin B_{12}, and other additions are detailed instructions for performing disc-inoculum and cup-plate microbiological assays.

The author has introduced minor modifications into some of the basal media and, in a number of assays, has changed the method of preparing the inoculum; instead of transferring the micro-organism to saline solution he dilutes the sub-culture with Ringer's solution. He recommends a change of micro-organism for four of the vitamin and four of the amino acid assays.

Chemists not widely experienced in microbiological assays can obtain through this book comprehensive instructions for the assay of B-vitamins and amino acids at very low cost, and expert microbiologists owning the earlier edition will probably be willing to spend the same small sum to learn of the author's later experiences and views, although these may not be fully in accord with their own. A. J. AMOS

Publications Received

EXPERIMENTS FOR YOUNG CHEMISTS. By E. H. COULSON, M.Sc., F.R.I.C., and A. E. J. TRINDER. Pp. 158. London: G. Bell & Sons, Ltd. 1963. Price 15s.

CHROMATOGRAPHIC METHODS. By R. STOCK, B.Sc., Ph.D., F.R.I.C., and C. B. F. RICE, B.Sc., F.R.I.C. Pp. viii + 206. London: Chapman and Hall. 1963. Price 40s.

APPLICATION OF DISTILLATION TECHNIQUES TO RADIOCHEMICAL SEPARATIONS. By JAMES R. DEVOE. Pp. vi + 29. Washington, D.C., U.S. Department of Commerce, Office of Technical Services. 1962. Price 50 cents.

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Lectures and papers presented at the Second Symposium on Chromatography organized by the Société Belge Des Sciences Pharmaceutiques held in Brussels, September 14th–15th, 1962.

CHARACTERISTIC FREQUENCIES OF CHEMICAL GROUPS IN THE INFRA-RED. By M. ST. C. FLETT. Pp. x + 98. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 25s.

ABSORPTION SPECTRA IN THE ULTRAVIOLET AND VISIBLE REGION. Volume IV. Edited by DR. L. LÁNG, with the collaboration of DR. A. BARTECKI, DR. J. SZÖKE, DR. G. VARSÁNYI and M. VIZESY. Pp. 414 (loose leaf) Budapest: Akadémiai Kiadó. 1963.

STANDARDMETHODEN DER PRAKTISCHEN CHEMIE: PRAPARATIVE METHODEN DER ORGANISCHEN CHEMIE. Part 2. GRUND-VERBINDUNGEN 1. Edited by ERNST POULSEN NAUTRUP. Pp. 58 (loose leaf). Braunschweig, Germany: Friedr. Vieweg & Sohn. 1963. Price DM 5.90.

CHEMICAL ANALYSIS. Edited by P. J. ELVING and I. M. KOLTHOFF. Volume XVII. ELECTRON PROBE MICROANALYSIS. By L. S. BIRKS. Pp. x + 253. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 70s.

VAPOUR PRESSURE OF THE ELEMENTS. By AN. N. NESMEYANOV. Translated and edited by J. I. CARASSO. Pp. vi + 469. London: Infosearch Limited, distributed by Cleaver-Hume Press Ltd. 1963. Price 105s.

CHEMICAL ANALYSIS. Edited by P. J. ELVING and I. M. KOLTHOFF. Volume XIII. ALTERNATING CURRENT POLAROGRAPHY AND TENSAMMETRY. By B. BREYER and H. H. BAUER. Pp. xx + 288. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 90s.

BRITISH NATIONAL FORMULARY: 1963. Standard Edition. Pp. 300. London: The British Medical Association and The Pharmaceutical Society of Great Britain. 1963. Price 10s.

PHYSICAL CHEMISTRY. By WALTER J. MOORE. Fourth Edition. Pp. xiv + 879. London: Longmans, Green and Co. Ltd. 1963. Price 50s.

Correction

THE "Report of the Proceedings of the Thirteenth Session of the International Commission for Uniform Methods of Sugar Analysis," listed under PUBLICATIONS RECEIVED last month (page 822) is only obtainable from Monsieur R. Saunier, Secretaire, I.C.U.M.S.A., Syndicat National des Fabricants de Sucre de France, 23, Avenue d'Iena, Paris XVI^{e}, France, and **not** from I.C.U.M.S.A., Keston, Kent, as indicated in the original entry.

Report of the Analytical Methods Committee: Reprints

Nitrogen Factors for Chicken

THE Report prepared by the Meat Products Sub-Committee, "Nitrogen Factors for Chicken," reprinted from *The Analyst*, August, 1963, **88**, 583–584, is now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1. Price to members, 1s. 6d. each; to non-members, 2s. 6d. each.

Reports of the Analytical Methods Committee are only available from the Secretary (not through Trade Agents) and remittances, made out to the Society for Analytical Chemistry, MUST accompany orders.

Reprints of Review Papers

"Circular Dichroism"

REPRINTS of the Review Paper, "Circular Dichroism," by R. D. Gillard, published in this issue of *The Analyst*, will soon be available from the Secretary, Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 2s. 6d. per copy, post free.

"Information Retrieval in the Analytical Laboratory"

REPRINTS of the Review Paper, "Information Retrieval in the Analytical Laboratory," by D. R. Curry, published in this issue of *The Analyst*, will soon be available from the Secretary, Society for Analytical Chemistry, at the above address, price 2s. 6d. per copy, post free.

A remittance for the correct amount, payable to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not available through Trade Agents.

Erratum

SEPTEMBER (1963) ISSUE, foot of p. 738, present address of T. Doran. *For* "Sunbury-on-Thames, Surrey." *read* "Sunbury-on-Thames, Middlesex."

DECEMBER, 1963 Vol. 88, No. 1053

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETINGS

An Ordinary Meeting of the Society was held at 3 p.m. on Thursday, November 7th, 1963, in the Wellcome Building, Euston Road, London, N.W.1.

The subject of the meeting was "Gas Analysis." At the afternoon session the Chair was taken by the President, Dr. D. C. Garratt, Hon. M.P.S., F.R.I.C., and the following papers were presented and discussed: "Some Recent Developments in Gas Chromatography and Infrared Gas Analysis," by D. W. Hill, M.Sc., Ph.D., F.Inst.P., A.M.I.E.E., A.R.I.C.; "Some Problems Associated with the Analysis of Town Gas by Means of Gas Chromatography," by G. R. Boreham, B.Sc., A.R.I.C.; "Some Methods for Moisture Determination in Gases," by J. H. Scawin; "The Use of the Electrolytic Hygrometer for the Determination of Moisture in Gases," by J. E. Still, B.Sc., F.R.I.C. The Chair at the evening session was taken by Dr. S. G. Burgess, B.Sc., F.R.I.C., F.Inst.Pet., and the following papers were presented and discussed: "Sonic Gas Analysers—Uses and Limitations," by A. E. Martin, Ph.D., D.Sc.; "Some Electrochemical Methods of Gas Analysis," by J. H. Glover, B.Sc., F.R.I.C.; "The Use of a Slow Injection Syringe for Calibration in the Volumes per Million Range," by H. F. Downing, B.Sc.; "Apparatus for Handling Gas Samples and for the Preparation of Gas Mixtures of Known Composition," by J. E. Still, B.Sc., F.R.I.C.; "Dispersive and Non-dispersive Infrared Analysers for In-line Measurements," by A. W. Hough-Grassby, B.Sc., Ph.D.

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, December 4th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. C. Garratt, Hon.M.P.S., F.R.I.C.

The subject of the meeting was "Differential Thermal Analysis" and the following papers were presented and discussed: "Differential Thermal Analysis and its Application to Soil and Plant Materials," by R. C. Mackenzie, D.Sc., Ph.D., F.R.I.C., F.G.S., F.R.S.E. (presented by B. D. Mitchell, B.Sc., A.R.I.C., and followed by a short film dealing with the method of differential thermal analysis as it is performed at the Macaulay Institute); "The Use of Differential Thermal Analysis in the Mineralogical Investigations of Building Materials," by H. G. Midgley, M.Sc., Ph.D., F.G.S.; "The Application of Differential Thermal Analysis to Polymeric Materials," by D. E. Eaves, B.Sc., Ph.D.

NEW MEMBERS

Ordinary Members

Ijere Uguru Agwu, A.I.S.T., M.R.S.H.; David Valentine Atterton, M.A., Ph.D.(Cantab.), A.I.M.; Mohssen Bavendi, Pharm.D.(Iran); Harry Leslie Blamires; Frank Arthur Chappell, B.Sc.(Lond.), A.Inst.P.; Philip John Cooper, B.Sc.(Lond.), A.R.I.C.; Terence Michael Duley; Charles Edward Dyer, M.P.S.; Fred Howcroft Harrison; John Michael Hibbs, B.Sc.(Lond.); Robert Paul Hirsch; Alexander Hood, B.Sc.(Glas.), A.Inst.P., F.R.I.C.; Meryl Ramsey Jackson, B.Sc.(Leeds); Michael Douglas Lack; Roy Basil Walter Lowndes; Lin Ma, B.Sc. (West China; State University of New York), Ph.D.(Leeds); Donald Frank Charles Morris, B.Sc., M.A., D.Phil.(Oxon.), F.R.I.C.; Eivor Eva Josefina Naddermier; Paolo Papoff; Gerald Russell, F.R.I.C., A.R.P.S.; John Edward Saunders, A.R.I.C.; Derek Bernard Schaverien, B.Sc.(Lond.), A.R.I.C.; James Scott, A.R.I.C.; Clemente Tarantola; Raymond Herbert Trust, L.R.I.C.; Robert Laurence Weston.

JUNIOR MEMBERS

John Robert Ellis; John Edward Ellithorne, Dip.Tech.(Birm.); John Barry Pickup; Iqbal Waheed Siddiqi, B.Sc.(Karachi); Robert Simpson; Brian Sims, B.Sc.(Hull); Roger Edward Weetman; Paul Young, Dip.Tech., A.C.T.(Birm.).

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Physical Methods Group with the Modern Methods of Analysis Group of the Sheffield Metallurgical Association was held at 7 p.m. on Tuesday, October 22nd, 1963, in the Conference Room of the British Iron and Steel Research Association, Hoyle Street, Sheffield, 3. The Chair was taken by the Chairman of the Physical Methods Group, Dr. W. Cule Davies, F.R.I.C.

The following papers were presented and discussed: "The Determination of Oxygen in Metals by Fast-neutron Activation Analysis," by A. L. Gray, B.Sc.; "High-pressure Plasmas as Emission Sources," by S. Greenfield, L.I.M., I. L. Jones, D.L.C., A.R.I.C., and C. T. Berry, A.R.T.C.S., A.R.I.C. (see summaries below).

THE DETERMINATION OF OXYGEN IN METALS BY FAST-NEUTRON ACTIVATION ANALYSIS

MR. A. L. GRAY described the technique of activation analysis and gave a short account of recent developments that made the technique practicable for routine plant analytical work. He discussed the convenience of the method for the determination of oxygen in metals by use of fast neutrons and described the development of a prototype automatic equipment, the "Analox."

The speaker described the assessment of the prototype instrument and discussed the correlation of the results obtained on a wide variety of samples with those by other methods, and outlined potentialities of the instrument. The paper concluded with a brief description of the fully developed version of the instrument and the considerations involved in its use in a steel works.

HIGH-PRESSURE PLASMAS AS EMISSION SOURCES

MR. I. L. JONES described high-pressure thermal plasmas and compared them with low-pressure plasmas. He discussed the production of both direct-current arc and radio-frequency induced plasmas. He then described the application of plasmas as emission sources for spectrographs and flame spectrophotometers.

The speaker discussed the sensitivity and variance and the absence of interference from non-volatile compounds (as compared with flame sources) of plasma sources on flame spectrophotometers. He mentioned future applications of these sources, including the possibility of the use of direct calibration methods (obviating the need for internal standards) in emission analysis and the introduction of powdered samples into the plasma.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, November 23rd, 1963, at the Old Nag's Head Hotel, Lloyd Street, Manchester. The Chair was taken by the Chairman of the Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

The following paper was presented and discussed: "The Chemistry of Wines and Spirits," by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, November 8th, 1963, at the Royal Society of Edinburgh, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. R. A. Chalmers.

The following paper was presented and discussed: "Atoms and the Analyst—Some Confessions of an Unqualified Chemist," by J. M. A. Lenihan, M.Sc., Ph.D., A.M.I.E.E., F.Inst.P.

A Joint Meeting of the Scottish Section with the Glasgow Section of the Society of Chemical Industry was held at 6 p.m. on Friday, November 22nd, 1963, at the Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Scottish Section, Dr. R. A. Chalmers.

The following papers were presented and discussed: "The Presentation of Scientific Papers," by J. D. Nisbet, M.A., B.Ed., Ph.D., F.B.P.S.; "Editing a Scientific Journal," by J. B. Attrill, M.A., F.R.I.C.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Thursday, November 14th, 1963, at the Lanchester College of Technology, Cox Street, Coventry. The Chair was taken by the Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Some Observations on the Use of Ion Exchange in Analytical Chemistry," by J. E. Salmon, B.Sc., Ph.D., F.R.I.C.

PHYSICAL METHODS GROUP

A Special General Meeting of the Group was held at 6.30 p.m. on Tuesday, September 10th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Dr. W. Cule Davies, F.R.I.C.

Mr. S. G. E. Stevens was elected Honorary Auditor in place of Dr. D. C. Garratt, who had ceased to be eligible on being elected to the Presidency of the Society.

BIOLOGICAL METHODS GROUP

An Ordinary Meeting of the Group was held at 7 p.m. on Wednesday, October 23rd, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. W. A. Broom, B.Sc., F.R.I.C.

The subject of the meeting was "Assay of Virus Vaccines" and the following papers were presented and discussed: "The Control Testing of Vaccines," by D. I. Magrath, B.A., Ph.D.; "Manufacture and Control of Poliomyelitis Vaccine," by A. J. Beale, M.D., Dip.Bact.

Thermogravimetric Analysis

A Review*

BY A. W. COATS AND J. P. REDFERN

(*Chemistry Department, Battersea College of Technology, London, S.W.*11)

SUMMARY OF CONTENTS

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Techniques
Kinetic studies
Apparatus
Applications—
    (*i*) Analytical chemistry
    (*ii*) Inorganic chemistry
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Conclusion

THERMAL methods of investigation, generally referred to as thermo- or thermal analysis or thermo-analytical techniques, have found wide application in recent years.[1] These may be defined as experimental methods for characterising a system (element, compound or mixture) by measuring changes in physico-chemical properties at elevated temperatures as a function of increasing temperature.[2] The two chief methods are (*a*) differential thermal analysis,[3] in which changes in "heat content" are measured as a function of increasing temperature and (*b*) thermogravimetric analysis, in which changes in weight are measured as a function of increasing temperature. Other methods that come within this definition involve the use of changes in gas volume or pressure; changes in solid volume; changes in electrical resistance; changes in ultraviolet, visible or infrared transmission or reflectance.[4]

The two techniques, (*a*) and (*b*), provide information relating to certain physical and chemical phenomena, which are listed below—

Physical Phenomena	Chemical Phenomena
Crystalline transition	Chemisorption
Second-order transition	Desolvation (especially dehydration)
Fusion	Decomposition
Vaporisation	Oxidative degradation
Sublimation	
Absorption	Solid-state reactions
Adsorption	Solid - gas reactions (*e.g.*, oxidation or reduction)
Desorption	

The basic instrumental requirements for thermogravimetric analysis are a precision balance and a furnace that is programmed for a linear rise of temperature with time. Thermogravimetry can provide information on all the phenomena listed above, except crystalline transitions, fusions and those solid-state reactions that occur without change in weight.

Although information can be obtained by carrying out the weighing operations manually, nowadays automatic continuous recording of the weight and temperature are usual; the continuous record of weight and temperature ensures that no features of the weight loss - temperature curve are overlooked. The results from a thermogravimetric run may be presented by—

(*i*) weight (corrected weight—see below on "Corrections", p. 908), *versus* temperature (or time) curve, referred to as the thermogravimetric curve, see Fig. 1 (*a*), or

(*ii*) Rate of loss of weight *versus* temperature curve, referred to as the differential thermogravimetric curve, see Fig. 1 (*b*).

In (*i*), the weight axis may be scaled in one of several ways, *e.g.*, (*a*) as a true weight scale, (*b*) as a percentage of the total weight, (*c*) as a percentage of the total weight loss or as a fraction of the total weight lost, (*d*) in terms of molecular-weight units, or (*e*) expressed in terms of α (where α = fraction decomposed). (When the molecular weight of the compound is known, method (*d*) affords a convenient method of plotting the results, since it is easy to extract data relating to lost fragments, for example, in the study of an inorganic complex—it also forms an easy method of comparison of a family of such compounds.) The use of method (*e*) should be limited to a single-stage process, *i.e.*, a special instance of (*c*).

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* Reprints of this paper will be available shortly. For details, please see p. 984.

The following features of the thermogravimetric curve may be identified—

(i) A horizontal portion or plateau, which is indicative of constant weight.

(ii) A curved portion; the steepness of the curve is indicative of the rate of weight loss, and this will obviously pass through a maximum, giving an inflection with $\frac{dw}{dt}$ as a maximum. The shape of the curve is dependent on the variables discussed in the section on "Techniques" (*vide infra*).

(iii) An inflection (at which $\frac{dw}{dt}$ is a minimum, but not zero) may imply the formation of an intermediate compound. It may, however, be due to disturbances in the heating rate or in thermocouple response. It is necessary to ensure a regular rise of temperature,[5] and therefore it is desirable to have an independent temperature - time record to ensure the reliability of the results.[6]

The portion of the differential thermogravimetric curve lying on the line $\frac{dw}{dt} = 0$, see Fig. 1 (*b*), is equivalent to the horizontal portion of the thermogravimetric curve. The peak of the differential thermogravimetric curve corresponds to the curved portion of the thermogravimetric curve, whereas the peak maximum of the former is identical with the point of maximum slope of the latter. A trough on the differential thermogravimetric curve corresponds to an inflection at which $\frac{dw}{dt}$ is a minimum on the thermogravimetric curve. The height of the trough above the line $\frac{dw}{dt} = 0$ affords some measure of the stability of the intermediate and the extent to which the two consecutive reactions (corresponding to the peaks on either side of the trough) overlap. The differential thermogravimetric curve offers certain advantages over the thermogravimetric curve in the matter of presentation. Features in the thermogravimetric curve that are not readily discerned are more clearly seen in the differential thermogravimetric curve, *e.g.*, any change in the rate of weight loss may be seen immediately

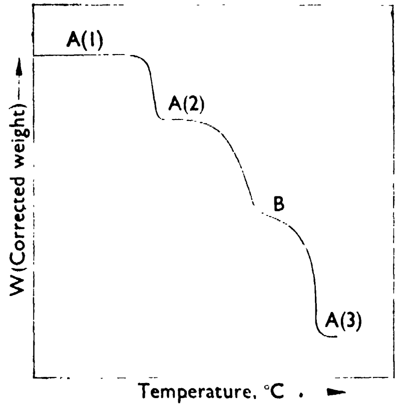

Fig. 1(*a*). Thermogravimetric curve: A (1), (2) and (3) are plateaux in the decomposition curve of the material. B is a point of reflexion (at which $\frac{dw}{dt}$ is a minimum)

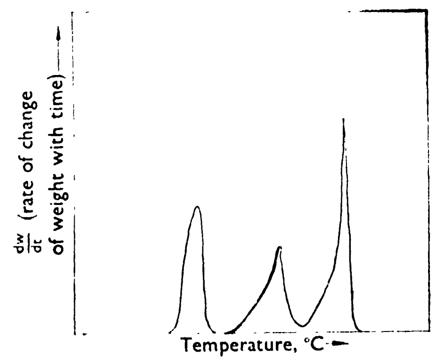

Fig. 1(*b*). Differential thermogravimetric curve: A (1), (2) and (3) correspond to the plateaux on the thermogravimetric curve (at which $\frac{dw}{dt}$ is zero). Trough B corresponds to the point of inflexion on the thermogravimetric curve (at which $\frac{dw}{dt}$ is a minimum) as a trough, indicating two consecutive reactions, or as a shoulder to the peak, indicating two almost overlapping reactions or as a tail to a peak, which is probably an indication of strong adsorption of the volatile product on the new solid phase.[7] Differential thermogravimetric curves often show considerable similarity to differential thermal analysis curves or permit a ready comparison to be made.[8,9,10]

The use of thermogravimetric results for evaluating thermal stability has focused attention on finding a workable definition for a suitable standard for describing decomposition. The temperature at which a reaction begins in any particular thermobalance run is dependent on many variables, of which the rate of heating is perhaps the most important. This temperature is neither a true decomposition temperature, below which the reaction rate is zero, nor is it a transition temperature.[11] In fact, there is often little correlation between results from isothermal runs and non-isothermal runs.[5] Newkirk[12] argues that knowledge of this temperature is useful and the term "procedural decomposition temperature" used by Doyle,[13] in his polymer studies, stresses the dependance of this temperature on the powerfully influential procedural details. Pellon[14] in his work on the stability of phosphorus-containing polymers uses a temperature T_{10} (a temperature at which the cumulative weight change reaches 10 per cent.) as a means of defining thermal stability. A comparison of decomposition temperatures has often led to controversy owing to a lack of appreciation that the value is a function of method, apparatus and procedure. A clear statement of conditions used should be stated when quoting decomposition temperatures. Guiochon[11] comments: "Thermogravimetric measurements are easy to carry out, but somewhat difficult to account for."

A survey of the history of thermogravimetric analysis up to 1940 has recently been published by Duval,[15] in which he traces the design and construction of the first thermobalance back to Nernst and Riesenfeld[16]; Wache[17] has written an article on Chevenard. The second edition of Duval's book, which is now available,[18] is divided into a discussion of (*a*) "The Thermobalance" and (*b*) "The Thermolysed Substances." Review articles have been published on recording balances and instrumentation[19,20] and on thermogravimetry.[21 to 27] Several symposia have been held, notably in the United States.[28,29] The inception of the first Thermoanalysis Institute held at the Fairleigh-Dickinson University, New Jersey,[30] occurred in 1962. A survey of literature, published about four times a year, commenced in May, 1962.[1]

TECHNIQUES

This section is concerned with two aspects of thermogravimetry. The first arises from the dynamic nature of the method, and the second is concerned with the factors influencing the shape of the thermogravimetric curve, and these must be taken into account to obtain meaningful and reproducible results, so that thermogravimetric data on different compounds can confidently be compared.

CORRECTIONS—

If a crucible, that is known not to change weight, is heated when empty, there is, generally, an apparent change in weight with increasing temperature (see Fig. 2), *e.g.*, a

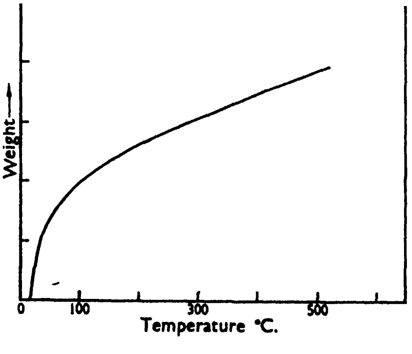

Fig. 2. Typical correction curve

crucible weighing 2 g would be expected to have an apparent change in weight at 1000° C of, say, 0·1 per cent. of the crucible weight; this would amount to an apparent change in weight of 2 per cent. if the sample weighed 0·1 g. This apparent weight change is caused by the interplay of a complex combination of several factors, such as air buoyancy, convection effects within the furnace, crucible geometry, radiation effects, the atmosphere in the furnace and the fact that the crucible support is subject to a temperature gradient within the furnace. It is necessary to use the apparent weight change of the empty crucible as a correction curve to arrive at the actual weight change occurring in a sample. This is considered to be more satisfactory than the use of a tare crucible, as has been proposed,[31] in a separate furnace, in view of the possible non-uniformity of the hot zones in the two furnaces.

Since several factors contribute to this correction curve it is found that the corrections to be applied differ with different rates of heating. Various workers[12,32,33] have discussed the factors affecting the correction curves, and Duval[34] and Newkirk[12] have suggested the use of vents at the top of the furnace to reduce the size of the correction. This has been criticised[32] as amounting to critical damping of the hot-zone volume. Empirical equations have been derived[35,36,37] for the correction curves, but because of the complexity of factors involved it seems simpler to use a correction curve determined practically under conditions identical to those of the actual experiment.

FACTORS AFFECTING THE RESULTS OBTAINED—

There are several factors that may, to a greater or less extent, influence the shape of a corrected thermogravimetric curve for a particular compound.

Heating rate—For a single-stage endothermic reaction, where T_1 is the procedural decomposition temperature and T_f is the temperature at which the reaction is completed, it has been shown[5,38,39,40] that—

(*i*) $(T_f)_f > (T_f)_s$,

where the subscripts *f* and *s* refer to a fast and slow rate of heating, respectively,

(*ii*) $(T_1)_f > (T_1)_s$ and

(*iii*) $(T_f - T_1)_f > (T_f - T_1)_s$.

Newkirk[12] has shown that, at any given temperature, the extent of decomposition is greater at a slow rate of heating than for a similar sample heated at a faster rate. If the reaction involved is exothermic, the sample temperature will rise above the furnace temperature; it has been shown (in unpublished work by us) that the difference between the furnace temperature and the sample temperature is greatest for the faster rate of heating when a reaction is occurring. When successive reactions are involved, the rate of heating may well determine whether or not these reactions will be separated. The appearance of a point of inflection in the thermogravimetric curve at a fast heating rate may resolve into a plateau at a slower heating rate.[23] The importance of heating rates was stressed by early workers[41,42] and has been much studied.[43 to 47]

Sample—The sample weight can affect the thermogravimetric curve in three ways—

(*i*) The extent to which endo- or exothermic reactions of the sample will cause the sample temperature to deviate from a linear rise; in general, all other factors being equal, we have found that the greater the weight of sample, the greater will be the deviation.

(*ii*) The degree of diffusion of the product gas through the void space around the solid particles; under static conditions, the environmental atmosphere (*i.e.*, the atmosphere immediately surrounding the reacting particles) will be somewhat governed by the bulk of the material in the crucible.

(*iii*) The possible unevenness of the temperature throughout the sample, particularly if it has a low thermal conductivity.

Thus the use of as small a weight of sample as possible, within the limits of the sensitivity of the balance, is to be preferred.

The state of sub-division of the sample is important. The use of large crystals may result in spitting, as described by C. J. Keattch in a private communication. The sample may foam and bubble.[48] Most thermogravimetric studies have been carried out on powders, and the effect of particle size or surface area has been widely studied.[41 to 52] The smaller the particle size the greater the extent to which equilibrium is reached, and at any given temperature the extent of decomposition is greater.

The pre-history of the sample is also important. Wendlandt[53] has discussed the effect of precipitation technique on the thermogravimetric curve of ammonium molybdophosphate. Recent work on magnesium hydroxide from different sources provides a good illustration of this factor.[54]

Crucible—The geometry of the crucible used will profoundly affect the shape of the thermogravimetric curve. Crucible design has been studied,[55,56] and the use of a crucible fitted with a piston (where reactions take place under a pressure of 1 atmosphere of the liberated gas) or an on open tray (at the partial pressure of the gas in the atmosphere) have been compared. This latter arrangement has been used by other workers.[57,58] The design of a crucible to overcome possible condensation on the support rod or decrepitation before decomposition has also received attention.[59,60] In certain instances the material of which the crucible is constructed affects the decomposition pattern.[61]

Atmosphere—

(*i*) *When the atmosphere does not take part in the reaction*—The use of an inert atmosphere in thermogravimetric analysis has been discussed.[59] The rôle of the gas is to remove gaseous products from the vicinity of the sample, to ensure that the environmental atmosphere is kept as constant as possible throughout the experiment and to prevent reaction occurring between the sample and air as normally employed,[62,63] or between the volatile components and the atmosphere.[5,59] Other workers[64 to 68] have used vacuum conditions to achieve the same results. The dependence of the shape of thermogravimetric curves on the pressure of the gas in the reaction chamber has been studied.[69]

(*ii*) *When the atmosphere is involved in the reaction*—Some work has been carried out with atmospheres either of humidified air or high pressure steam[12,70,71]; a dynamic air atmosphere has been used to study the roasting of copper sulphide[43] and the oxidation of reduced iron catalyst.[72] A comparison of some six atmospheres in metal - gas reactions has been made by Markowitz and Boryta.[73,74] Reducing atmospheres, *e.g.*, hydrogen, have been used.[75,76,77] Other atmospheres, in which reactions occur between the gas and the sample, that have been studied include hydrogen sulphide[78] and carbon dioxide.[79]

KINETIC STUDIES

Thermogravimetric data can be used to evaluate kinetic parameters of reactions involving weight loss (or gain) of the following four types—

$$\begin{aligned} &A_s \longrightarrow B_s + C_g{}^{80,81}\\ &A_s + B_s \longrightarrow C_s + D_g\\ &A_s + B_g \longrightarrow C_s{}^{73}\\ &A_s \text{ or } A_l \longrightarrow A_g{}^{82}\end{aligned}$$

The advantages of determining kinetic parameters by thermogravimetric methods rather than by conventional isothermal studies are (*i*) considerably less data are required than in the isothermal method, (*ii*) the kinetics can be probed over an entire temperature range in a continuous manner and (*iii*) when a sample undergoes considerable reaction in being raised to the required temperature, the results obtained by an isothermal method are often questionable. To these reasons may be added the advantage of using one single sample in the study.

It is important to know the temperature accurately and to ensure that endo- or exothermic effects do not cause the rate of heating to depart from its constant value.[73] The use of small samples, within the limits of sensitivity of the balance, is therefore necessary. It should be remembered that kinetic parameters derived from thermogravimetric experiments are dependent on the procedural details, *e.g.*, crucible shape and material, particle size, prehistory of sample and heating rate.[47,54]

The earliest attempt to use thermogravimetric curves for kinetic data appears to have been made by Van Krevelen, Van Heerden and Huntjens.[83] They derived an approximate equation, from which it was possible by graphical methods to determine the order of reaction (they differentiated between orders of 0, 1 and 2). They studied the pyrolysis of coal and showed that the primary decomposition was first order. On this assumption they developed graphical methods for determining the activation energy and frequency factor, knowing the rate of heating, temperature of maximum rate of decomposition and the half-value width of the differential thermogravimetric curve.

Kaesche-Krischer and Heinrich[47] used a similar approach in their study of the pyrolysis of poly(vinyl alcohol) in a vacuum, and Schnitzer, Turner and co-workers[84,85] studied the organic matter of Podzol soils. Turner, Hoffman and Chen[54] modified Van Krevelen's approach to accommodate a two-thirds order in their work on the thermal decomposition of magnesium hydroxide.

Freeman and Carroll[80] derived the equation—

$$-\frac{E/2{\cdot}3\mathrm{R}\Delta T^{-1}}{\Delta\log W_{\mathrm{r}}} = -x + \frac{\Delta\log(dw/dt)}{\Delta\log W_{\mathrm{r}}} \quad .. \quad .. \quad .. \quad (1)$$

where E = activation energy, R = general gas constant, T = absolute temperature, x = order of reaction and $W_{\mathrm{r}} = (W_{\mathrm{c}} - W)$, where W = weight loss at time t, and W_{c} = weight loss at completion of the reaction. This equation was derived by assuming a rate expression—

$$-\frac{dX}{dt} = kX^{x} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where X = amount of A at time t and the rate constant, k, is given by the simple Arrhenius expression—

$$k = Ze^{-E/RT} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where Z = frequency factor. Either by plotting a graph of $\frac{\Delta\log(dw/dt)}{\Delta\log W_{\mathrm{r}}}$ *versus* $\frac{\Delta T^{-1}}{\Delta\log W_{\mathrm{r}}}$ or by other suitable rearrangement of equation (1)[64,81,86] it is possible to derive values for both E and x.

This derivation has been used to study several dehydration reactions.[7,58,80,81,87 to 91] Barrer and Bratt[92] have used an adaptation of equation (1) in their study of non-stoicheiometric hydrates. The decomposition reactions of calcium oxalate,[80,81] thorium, lanthanum and uranium oxalates,[93] calcium carbonate,[80,81] chromium arsenate[58] and 12-tungstomanganic acid[94] have all been studied. Bear and Wendlandt[95] have studied the effect of added salts on the decomposition of trisethylenediamine and trispropylenediamine chromium$^{\mathrm{III}}$ chlorides and thiocyanates. Similarly, the pyrolysis of several polymers has been studied[64,86,96,97] However, several disadvantages appear to exist in the use of this derivation—

(*i*) in most practical instances the derivation seems to apply to an extremely limited portion of the decomposition curve;

(*ii*) there is considerable difficulty in obtaining a reliable value for the order of reaction;

(*iii*) none of the other methods of applying the derivation seems to overcome the difficulties; and

(*iv*) in certain reactions the method does not seem to yield an answer at all[87]—this may be due to the reaction itself and not inherent in the derivation.

In his method,[12] Newkirk assumes that all pyrolytic decompositions are first order, and his method involves calculating the first-order rate constants for a large number of temperatures, and then constructing the Arrhenius graph. In his study of Mylar (a plastic marketed by Du Pont Company Ltd.) he showed that the slope of the graph was independent of heating rate, but, as the rate of heating was increased, the graphs were displaced towards lower values of $1/T$, compared with the results obtained from isothermal studies. Smith[98] has used a similar approach to derive E_{p} (E_{p} denotes that E, the activation energy, may be somewhat dependent on the experimental procedure) for various polymers.

Other workers[44] have determined apparent activation energies by plotting the reciprocal of the final decomposition temperature against the logarithm of the heating rate.

Doyle[82] integrated a combination of expressions equivalent to equations (2) and (3), using an approximation. His technique is essentially one of curve fitting. He studied the zero-order volatilisation of liquid octamethylcyclotetrasiloxane and the first-order pyrolytic volatilisation of polytetrafluoroethylene. He confines himself to zero- or first-order reactions, but his approach cannot be used when the order is unknown.

Horowitz and Metzger[99] have recently developed another approach based on integration of a combination of expressions equivalent to equations (2) and (3) by an exact integral, using the substitution $(T - T_{\mathrm{s}}) = \theta$ where T_{s} is the temperature at which $\frac{W}{W_0} = \frac{1}{e}$, ($W$ = weight of sample at time t, and W_0 is the initial weight of the sample). The theory shows that a plot of $\ln (\ln \frac{W_0}{W})$ *versus* θ should be a straight line of slope $\frac{E}{RT_s^2}$. These workers have studied four polymers and the dehydration of calcium oxalate monohydrate. In each reaction they assume a particular order of reaction. For an unknown order they suggest the use of the position of maximum rate since this is governed by the order. It is, however, not always easy to determine the position of maximum rate with accuracy.

Coats and Redfern[100] used a different approximation for integrating a combination of equations (2) and (3). The graph of

$$\log \frac{1 - (1 - \alpha)^{1-n}}{(1 - n)T^2} \text{ versus } \frac{1}{T}$$

is a straight line of slope $-E/2{\cdot}3R$ for the correct value of n (α = fraction decomposed and n = order of reaction).

The activation energy can have a real meaning in solid-state kinetics, corresponding to the rate-determining step or steps, which might be the diffusion of the gaseous product out of the solid, or the transport of a particular ion, or the breakage of bonds. The order of reaction cannot presumably have the meaning attributed to it, as in a gas reaction, and may best be considered as a mathematical factor in the derived equations. However, geometric models of solid systems that lead to orders of reaction of $\frac{1}{2}$ and $\frac{2}{3}$ can be set up[101]; orders of 0 and 1 can also be justified.

Two points remain. The first is a plea for the use of standard symbols in the description of kinetic processes (see, for example, Garner[102]). The second is that it seems unlikely that the simple rate expression $\frac{d\alpha}{dt} = k(1 - \alpha)^n$, from which all the derivations quoted are ultimately derived, will be applicable to all solid-state decomposition reactions.

The use of computational methods for analysing thermogravimetric data is probably in wider use than the number of papers on this subject would suggest.[100,103] The advantages that these methods possess in assisting in the evaluation of kinetic parameters are obvious; their more widespread introduction will undoubtedly lead to a better coverage of some of the necessary studies on the effect of the variables, listed under "Techniques," on kinetic parameters.

APPARATUS

The comprehensive review of Gordon and Campbell[19] covered the whole field of automatic and recording balances up to 1959. Many of these are directly applicable or readily adaptable for use in thermogravimetry. Lewin[20] discusses six makes of commercial thermobalances on the market in 1962 and lists another four firms manufacturing thermobalances. Duval[18] puts the number of thermobalance models on the market at fifty-two. Other firms[104,105,106] known to us are manufacturing thermobalances.

The essential components of a modern thermobalance are: (*i*) balance, (*ii*) furnace, and (*iii*) recorder. It is desirable to have a reaction chamber to permit work to be carried out under a wide variety of conditions, *e.g.*, inert, oxidising or reducing atmospheres or under vacuum, and to permit gas analysis to be carried out. The balances used can be grouped into two types. They are the null-point and the deflection types of instruments. The former incorporates a suitable sensing element that detects any deviation of the balance beam and the application of a restoring force, proportional to the change in weight, to return the beam to its original null-point. This restoring force is then recorded either directly or through a transducer. These null-point type instruments are often readily adaptable to working under vacuum conditions. Deflection instruments, *e.g.*, based on a conventional analytical balance, a helical spring, a cantilever beam, a strain gauge or a torsion balance, involve the conversion of deviations into a record of the weight change. The principles used in detecting and restoring deviations (in null-point balances), and in recording the changes in weight have been fully discussed.[19]

Furnace design and control is of great importance; it must be designed to provide a suitable smooth input so that it can maintain a linear heating programme or a fixed temperature, independent of any changes in external conditions. The comments on temperature control in differential thermal analysis[3] apply equally to thermogravimetric analysis. Control is generally achieved via a thermocouple situated as close to the furnace winding as possible.

The appearance of thermal pulsing effects owing to the periodic application of relatively large power increments leads to a ragged weight record,[32] and the use of some form of stepless control of power supply to the furnace through a saturable reactor is to be preferred.[107] Nichrome winding permits a maximum temperature of around 1100° C; platinum - rhodium winding permits a maximum of around 1450° C. Higher temperatures can be achieved by using a graphite tube furnace, but the associated control and measurement of temperatures raise considerable problems.

The recording system should be able to record both temperature and weight continuously and to make a periodic record of the time. The use of "$X_1 - X_2$"[105] or side-by-side[108] recording is to be preferred to the use of "X — Y" recording,[5] since these methods provide an independent record of both temperature and weight.

The prospective user has a variety of instruments available over a wide cost range. His choice should reflect the requirements of his particular field of study and the desiderata for good thermobalance design (see, for example, Lukaszewski and Redfern[23]), namely that—

(*i*) The thermobalance should be capable of continuously registering the weight change of the sample studied as a function of temperature (and time).

(*ii*) The furnace should be capable of reaching the maximum temperature desired. Most commercial instruments are marketed with a maximum of around either 1100° or 1500° C (some thermobalances are available and have been designed to work up to 2200°[105] or 2700° C[109]).

(*iii*) The rate of heating is truly linear and is reproducible.

(*iv*) The hot zone of the furnace is as near uniform as possible, and that the crucible is always located within this hot zone This is particularly important in deflection instruments, since the crucible will move in relation to its initial position in the hot zone during the course of the run.

(*v*) The instrument is as versatile as possible, providing for—

  (*a*) variation in heating rate,
  (*b*) heating in a dynamic flow of a controlled atmosphere, inert, oxidising or reducing,
  (*c*) heating *in vacuo*,
  (*d*) variation in chart speed to aid interpretation and
  (*e*) the possibility of carrying out accurate isothermal studies.

(*vi*) Physical effects (*e.g.*, radiation and convection currents, and magnetic effects caused by the winding of the furnace) due to the functioning of the apparatus do not upset the balance mechanism. No interaction should occur between conducting or magnetic samples being studied and the furnace winding. Possible chemical attack, from the gases used or the gases evolved, can be eliminated either by the design of the reaction chamber or by the materials used. The balance mechanism is sufficiently protected from the furnace, so that its sensitivity remains constant throughout the whole of the experimental run.

(*vii*) The temperature of the sample is measured as accurately as possible.

(*viii*) The sensitivity of the balance mechanism allows the study of small samples.

Several workers have described the construction of relatively simple inexpensive instruments, *e.g.*, those suitable for teaching the principles of the subject or for preliminary studies.[22,110,111,112] Micro-recording,[113,114] vacuum or controlled-atmosphere[112,114,115,116] thermobalances have been described in detail. Recent papers, in which automatic thermobalances[117 to 121] are described, include details of models capable of operating up to 40[117] and 60[118] atmospheres. In view of the trends in current instrument design reviewed by Eastwood,[122] the development of an automatic digital-recording thermobalance is of great interest.[123] There is considerable activity in the development of instruments capable of performing both thermogravimetric and differential thermal analysis either on the same sample or under similar conditions.[46,107,124,125,126] However, it should be borne in mind that the conditions for producing meaningful results are not necessarily the same for both techniques, although comparison is often valuable and, in some instances, essential.

Several workers have described modifications to existing commercial recording balances or thermobalances. Thus the Ainsworth recording balance has been incorporated in a thermobalance design[31]; the Stanton thermobalance has been adapted for work in atmospheres of hydrogen[127] and for producing the differential thermogravimetric curve.[10]

It is possible to measure the sample temperature directly without affecting the performance of a conventional balance by means of extremely fine connecting wires (of about 0·001-inch diameter) between the balance arm and a suitably located terminal block. The thermocouple wires then run up the support rod the crucible, and the bead may be located in or close to the sample, depending on the design of the crucibles used.[46,108]

APPLICATIONS

ANALYTICAL CHEMISTRY—

The widest application of thermogravimetric analysis to date has been in the investigation of analytical procedures: (*i*) in investigating suitable weighing forms for many elements; (*ii*) in testing materials that are actual or potential analytical standards; and (*iii*) in the direct application of the technique to analytical determinations.

Kobayashi[128] has reviewed the work of the Japanese School from 1925 to 1940 in the field of gravimetric analysis; from some twenty-seven references he listed about 300 precipitates and gave the recommended drying temperatures. In the first edition of his book,[129] Duval reported his studies on over 1000 gravimetric precipitates for nearly 70 elements. He concluded that only about 200 of these are suitable weighing forms for the elements. Erdey's book[130] on gravimetric analysis includes thermogravimetric, differential thermogravimetric and differential thermal analysis curves obtained on the Derivatograph[9] for each weighing form discussed. Two reviews have been published.[131,132]

There has been criticism of some of Duval's work on a number of grounds. Differences between other published work and that of Duval and his co-workers has been ascribed to different precipitating techniques,[53] to different washing techniques,[133] and to the use of high rates of heating.[134] Duval's criterion for rejecting a particular gravimetric precipitate as not being a suitable weighing form was that it did not give a plateau when heated at one particular rate of heating. Newkirk[12] refers to the investigation of zinc monosalicylaldoxime in which de Clercq and Duval,[135] using the method of Flagg and Furman[136] to prepare the precipitate, found no plateau on the thermogravimetric curve corresponding to the anhydrous compound. Detailed work by Rynasiewicz and Flagg[137] showed that the anhydrous form was stable from ambient temperature up to 285° C; further, that the plateau depended on the initial water content. Newkirk continues: "In as much as Duval has used a higher heating rate (ca. 380° C per hour), it is perhaps not surprising that he failed to find a plateau. The rejection of this precipitate as an analytical method for the determination of zinc on the grounds that it does not give a stable horizontal in the thermobalance at one particular rate of heating is clearly unwarranted. . . . The results suggest that when the thermobalance is used to study the drying of bulky precipitates that contain considerable water, it would be well to use very slow rates of heating."

The use of thermogravimetric data to interpret the best drying temperature (a constant temperature) must be made with a clear understanding of the dynamic nature of the technique. Regard must also be paid to all the factors mentioned in the section on "Techniques." It is clear that failure to do this may lead to considerable difficulty and controversy. To summarise—

(*i*) The lack of the appearance of a plateau, at one particular heating rate, is insufficient evidence on which to judge the suitability or otherwise of a particular weighing form.

(*ii*) The appearance of a plateau is not conclusive evidence that the weighing form is isothermally stable at all or any of the temperatures that lie on the plateau.[5]

(*iii*) It is evident that the most reliable information will be gained by using several different heating rates or, at least, a slow rate of heating, possibly with preliminary air drying.

However, there is much to be gained from a thermogravimetric investigation of a weighing form since the recommended temperatures for some procedures have been arrived at arbitrarily, and have frequently been quoted in the literature without any critical evaluation of the conditions required. Beamish and McBryde[138] have shown that certain instructions given for the heating or ignition of materials in some gravimetric determinations were quite inadequate.

Recent investigations include the study of: ammonium molybdophosphate[53] and quinolinium molybdophosphate[139] as suitable weighing forms for phosphorus; several sulphides[140] and selenides[141] to confirm methods of drying and weighing; lanthanum carbonate[142] and lanthanum oxalate[143] to find suitable weighing forms for lanthanum; the use of *m*-nitrobenzoic acid as a precipitant for cerium^{IV} [144]; and various compounds of plutonium to find suitable weighing forms for it.[145,146] Picrates and styphnates of some organic bases, such as guanidine and related compounds, have been examined since these derivatives are constantly used in characterisation and analysis.[147]

Thermogravimetric studies have been made of many substances of use or of potential use as standards in analysis. Thus a series of papers by Duval has appeared on the use of this technique in conjunction with infrared spectra.[148,149] Investigations of potassium hydrogen phthalate,[150] ethylenediaminetetra-acetic acid (EDTA) and its derivatives,[151] some tetraphenylboron salts of oximes[152] and 5-substituted barbituric acids[153] have been carried out.

It has been shown that soils can be analysed thermogravimetrically for determining hygroscopic moisture, organic matter, and inorganic carbonates.[154,155,156] Dupuis and Dupuis[157] have used thermogravimetry for determining calcium and magnesium in dolomite rock. The use of thermogravimetry in the study of the composition of non-interacting binary mixtures has been outlined.[23] Erdey and co-workers,[158,159] using the Derivatograph, have determined calcium, strontium and barium in a single sample by their precipitation as the mixed hydrated oxalate, using the losses of weight of extraneous water and water of crystallisation, and loss of carbon monoxide and dioxide. Berlin and Robinson have used thermogravimetry in their determination of magnesium, potassium and lead with dilituric acid[160] and for determining ethylenediamine and quinine,[161] with the same precipitant. Fluorine has also been determined thermogravimetrically.[162]

INORGANIC CHEMISTRY—

In recent years the greatest number of papers on thermogravimetric analysis has appeared within the field of inorganic chemistry. In a review of this nature it is not possible to discuss the work in detail; however, an attempt has been made to collate recent work on the basis of the anion of the compound studied. Studies of inorganic compounds are concerned with their stability, decomposition and structure. The types of reaction that have been observed are—

(*i*) Loss of constituent water molecules; such dehydrations may be either single or multi-stage, and may involve the loss of the elements of water, *e.g.*, from hydroxyl groupings.

(*ii*) Decomposition reactions, which may be either of a disproportionation or of a degradation type. Brill,[163] in what was probably the first publication in the field of thermogravimetry, presented data of this degradation type. He studied the decomposition of magnesium, calcium and barium carbonates—

$$M^{II}CO_3 \longrightarrow M^{II}O + CO_2.$$

(*iii*) Degradation reactions specifically involving the atmosphere, for example, oxidative degradation. An example is provided by the oxidation in air of trispropylenediamine chromium^{III} chloride—

$$2[Cr(pn)_3]Cl_3 \xrightarrow{air} Cr_2O_3 + \text{volatiles.}^{164}$$

(*iv*) Loss of constituent volatile ligands from inorganic complexes. An example is provided by the loss of ethylenediamine from trisethylenediamine chromium^{III} chloride—

$$[Cr(en)_3]Cl_3 \longrightarrow [Cr(en)_2Cl_2]Cl + en.^{95}$$

Though it has been clearly demonstrated that the thermal decomposition of inorganic compounds is dependent on the nature of both anion and cation, there has been little attempt to relate thermal decomposition data to modern theories in inorganic chemistry. Wendlandt[165] found a linear relationship between the ionic radii of the alkali metal ions and the temperature at which their tetraphenylboron salts began to decompose. Others have proposed reaction schemes to account for the pattern of thermal decomposition.[58,95,166,167]

Anions derived from elements of Group III—Some borates of lithium and sodium have been studied.[168] The decomposition of some calcium aluminate hydrates[169] and calcium carbo-aluminate[170] has been investigated, that of the former under constant water vapour pressure.

Anions derived from elements of Group IV—

(*i*) *Carbonates*—Freeman[81] has studied the kinetics of the decomposition of calcium carbonate, comparing results obtained with results from isothermal studies. Lanthanum carbonate has been investigated as a suitable weighing form for lanthanum[142]; other lanthanon carbonates have been studied.[171,172] The effect of crucible design on the decomposition characteristics of lead carbonate has been studied.[173] Other recent studies on carbonates include ammonium scandium carbonate[174] and certain cobalt^{III} complex carbonates.[175] Thermogravimetric studies of solid-state reactions between cerium oxide, neodymium oxide or samarium oxide with either sodium carbonate or sodium sulphate,[176] and of the strontium carbonate - zirconium oxide and barium carbonate - zirconium oxide systems have been made.[177] Wilburn and co-workers[8,178] have studied the systems sodium carbonate - silica and calcium carbonate - silica, by both thermogravimetric and differential thermal analysis and discussed their behaviour in relation to glass manufacture.

(*ii*) *Formates, acetates, oxalates and other oxycarbon anions*—Copper^{II} formate[179] and aluminium acetate[180] have recently been investigated. However, oxalates have received a great deal of attention, *viz.*—those of copper,[67] beryllium,[181] magnesium,[182] calcium[5,80,81] zinc,[67] cadmium,[67] lead,[67] manganese^{II},[67] cobalt,[173] nickel,[67,182] scandium,[183] yttrium,[183] lanthanum,[68,93,143,183] other lanthanons,[68,172,184,185] thorium[93] and uranium,[93,186] complex cobalt oxalates,[175] oxalato-niobates[187] and other complex oxalates.[188] The additivity of the decomposition curves of a mixture of oxalates has been demonstrated,[182] and the thermal decomposition pattern of oxalates in different atmospheres has received attention.[5,189] Other compounds of oxycarbon anions that have been studied include potassium hydrogen phthalate,[190] caesium propionate, butyrate, and isovalerate[191] and lanthanum and cerium^{III} palmitate, laurate and stearate.[192]

(*iii*) *Silicates*—The dehydration behaviour of several silicates and silicate minerals has been investigated.[193 to 198] The use of thermogravimetry combined with infrared studies has been suggested to permit a distinction to be made between constitutional and adsorbed water.[199] The effect of alkaline-earth chlorides on the dehydration of silica Xerogel has been studied.[200]

(*iv*) *Others*—Thermogravimetry has been used to determine the conditions under which calcium orthoplumbate^{IV} is formed.[201]

Anions derived from elements of Group V—

(*i*) *Nitrogen*—The reaction between boron oxide and sodamide to yield boron nitride[202] and the oxidation of aluminium nitride[203] have been studied thermogravimetrically. The thermogravimetric behaviour of cerium^{III},[204] praseodymium, neodymium, samarium,[172] thorium,[205,206] plutonium[146] and nickel[207] nitrates has been investigated. Lead azide has been studied.[208]

(*ii*) *Phosphorus*—The resistance to oxidation of some transition metal phosphides[209] has been described. Phosphates of ammonium,[210] sodium,[211] beryllium,[212] calcium,[213,214] strontium,[214] cadmium,[214] aluminium,[215,216] antimony,[217] chromium[166] and iron,[215,216] and some halophosphates have been studied.[218,219,220] The decomposition of disodium dihydrogen pyrophosphate[221] and the reactions between magnesium pyrophosphate and strontium oxide or magnesium hydroxide[222] have been reported. Gerrard, Mooney and Rothenbury have used thermogravimetry in their investigation of polymers formed from chloroborazoles and phosphorus esters.[223]

(*iii*) *Other anions*—Arsenic acid[224] and arsenates of chromium,[58] cobalt[225] and nickel[225] have been investigated thermogravimetrically. Ammonium metavanadate,[226] some niobates and tantalates[227] and some oxalato-niobates[187] have also been studied.

Anions derived from elements of Group VI—

(*i*) *Oxides, peroxides and related compounds*—Thermogravimetric studies of oxides may be sub-divided into two main sections: (*a*) Dehydration reactions of hydrated oxides and subsequent disproportionation reactions—

$$M_xO_y.nH_2O_{(s)} \longrightarrow M_xO_{y(s)} + nH_2O_{(g)}$$ [7,228 to 235]

$$M_xO_{y(s)} \longrightarrow M_xO_{y-z(s)} + \frac{z}{2}O_{2(g)}$$ [229,230,232,236]

(*b*) Solid-state reactions, which may be either solid - solid reactions involving loss of volatiles,[8,176,177,178,202,222,237,238,239] or solid - gas reactions involving loss or gain in weight,[76,240,241] have also been reported.

The thermal dehydration of copper,[242] beryllium,[181] magnesium,[54] iron^{III}[243] and nickel[193] hydroxides has been investigated thermogravimetrically. On the basis of thermogravimetric and infrared methods several metal oxyhydrates have been classified into four groups.[244]

(*ii*) *Sulphides, sulphates and other anions of sulphur*—The oxidation and thermal decomposition of copper,[43,245] zinc,[245] cadmium,[245] mercury,[245] germanium,[246] tin,[245] lead,[245] and various nickel sulphides[247,248,249] has been described, and there have been two studies on pyrites.[50,250] Sulphates of the metals listed below have been studied: copper,[245,251] beryllium,[181] magnesium,[252] calcium,[253] zinc,[245] cadmium,[245,254] mercury,[245] indium,[255] tin,[245] lead,[245] nickel,[256] uranium,[257] lanthanum,[258,259] and other lanthanons,[172,259] as well as several double and complex sulphates.[252,260 to 263] Solid - solid[264] and solid - gas[265] reactions involving sulphates have also been reported. Rocchiccioli[266] has studied the decomposition of sulphamic acid and some metal sulphamates.

(*iii*) *Anions derived from the other elements of Group VI*—The selenides of arsenic, rhenium and mercury have been shown to sublime at definite temperatures.[141] The decomposition of magnesium selenates has been compared to the corresponding sulphates.[252] The thermal decomposition of neodymium and basic yttrium chromate has been studied,[267] as well as some isopolychromates of potassium.[268] Tungstic acid,[269] ammonium paratungstate,[269] several 12-heteropoly tungstates,[89,90,94] ammonium[53] and quinolinium molybdophosphate[139] have been investigated thermogravimetrically.

Anions derived from elements of Group VII—Thermogravimetric studies of mono-amine dichloro zinc[270] and basic aluminium chlorides[271] have been made. A study of the application of high temperature thermogravimetry of chlorides and sulphates to soil analysis has been reported.[272] $CdI_2.xNH_3$[273] and some tetra-iodomercurates[274] have been investigated. The thermal decomposition of the following compounds involving oxyanions has been reported: sodium chlorite,[275] barium bromate[276] and chlorate[276] and copper^{II} iodate,[276] together with the perchlorates of potassium,[48] magnesium,[277] calcium[277] and barium.[276,277] Binary systems of potassium perchlorate with either alkaline or alkaline-earth metal nitrates,[278] and the course of the reaction between potassium chlorate and manganese dioxide[279] have been followed by using thermogravimetric techniques.

Barium permanganate[280] and ammonium perrhenate[281] and the compounds $AgMn_2O_4$ and Ag_2MnO_2[282] have been studied by using a thermobalance.

Anions derived from elements of Group VIII—Alkali metal,[283] copper,[284] magnesium[285] and alkaline-earth[285] ferrocyanides have been examined by Seifer. Work on ammonium chloroplatinate[286] and bromoplatinic acid[287] has also been reported.

Complex inorganic compounds—Complex compounds of cobalt, chromium, nickel and platinum in which the ligand groups are wholly or partly either ammonia, ethylenediamine, propylenediamine or pyridine have been widely studied by Wendlandt and his school[95,175,288 to 292] and also by others.[167,293,294]. The 8-hydroxyquinolines of many bivalent metals,[295,296] uranium[297] and plutonium[145] have been studied. Bivalent metal anthranilates,[298] substituted anthranilates of lanthanum and lead,[299] and other related chelate complexes[88,300,301] have all been studied thermogravimetrically. Other work on complexes has been carried out by Wendlandt and co-workers,[302,303,304] Charles[305] and Dhar and Basolo.[306] Clathrates of the type $Ni(CN)_2.NH_3\,X$, where X = benzene, thiophen, pyrrole, furan or phenol,[307] have been examined by using a quartz-spring thermobalance.

ORGANIC CHEMISTRY—

The application of thermogravimetric analysis to organic compounds is best discussed under two headings.

Synthetic organic polymers—These have been much studied in recent years, particularly with the advent of apparatus in which the sample can be heated in an inert atmosphere or under vacuum. Since the breakdown of polymers into the volatile monomer units is nearly always a simple single-stage process, these reactions have been used as standards in non-isothermal kinetics (see "Kinetic Studies").[64,82,96,97,99] H. C. Anderson has compared differential thermogravimetry with differential thermometry as a method for studying the pyrolysis of polymers.[308] Recently Doyle[309] has derived equations from which it is possible to determine the isothermal life of polymers from thermogravimetric data. Table I lists some polymers that have been studied by a thermogravimetric technique.

TABLE I

SYNTHETIC POLYMERS STUDIED BY THERMOGRAVIMETRY

Polymer	References—	
	Kinetics studied	Kinetics not studied
Epoxide polymers	97	65, 310
Polyamides	311	—
Polyethylene	64, 99	312
Polymers based on methyl methacrylate	99	52
Polypyromellitimides	—	63
Polystyrene	64	—
Poly(t-butyl acrylate)	313	—
Polytetrafluoroethylene	82, 96	310, 314
Poly(vinyl alcohol), PVA	47, 315	—
Poly(vinyl chloride), PVC	316	62, 310
Other vinyl polymers	—	317
Phenolic polymers	—	318 to 320
Silicones	—	310
Urea - formaldehyde resins	—	321

Other organic compounds—Few simple organic compounds have been studied. Duval[322] has investigated the thermal stability of some organic compounds used as analytical standards. Other compounds studied include EDTA,[151] barbituric acid and related compounds.[153] Some organo-tin compounds have been investigated by thermogravimetry.[323] The pyrolysis behaviour of several coals, peats and bitumens has been studied[324 to 327]; their kinetics of decomposition has also received attention.[83,328] The mechanism of thermal decomposition of some organo-montmorillonites has been investigated.[329] Thermogravimetric and differential thermal analytical studies have been made of the pyrolysis of wood and of wood treated with inorganic salts. Some conclusions regarding the action of salts that are flame retardant are discussed.[330,331] There have been two studies on inclusion or clathrate compounds. McAdie[332] has studied the urea - n-paraffin inclusion compounds, and Gilford and Gordon[67] have examined the behaviour of some quinol clathrates under ambient and reduced pressures.

COMPLEMENTARY PROCEDURES

We have reviewed the various applications of thermogravimetric analysis, but there has been little stress on the fact that, in many of the papers referred to, other techniques have been used to complement the information gained from thermogravimetry. In certain investigations, it is essential to use a complementary technique if the interpretation of the course of a thermal decomposition is not to be pure guess-work; at least, chemical analysis of the solid removed from the thermobalance at an appropriate point on the thermogravimetric curve[58] or, alternatively of the evolved gas[316] should be carried out. Information gained from thermogravimetry becomes more meaningful or can readily be extended by the use of techniques that are applicable to solid-state chemistry.[25] It is not the object of this review, however, to discuss these procedures in detail but to offer a few pertinent comments.

DIFFERENTIAL THERMAL ANALYSIS—

This method is taken first, owing to its close affinity to thermogravimetry (see Introduction, p. 906). Both are dynamic methods, and both are very much dependent on procedural details.[3] It is not true that what is good technique for thermogravimetric analysis is necessarily so for differential thermal analysis. There is no doubt that much useful and meaningful information can be obtained with equipment designed to carry out both thermogravimetry and differential thermal analysis at one and the same time on the same sample—the success of the Derivatograph[9,50,158,159,210,243] is proof of this. Nevertheless, there are limitations. For example, in differential thermal analysis the sample is generally packed down in the crucible[3] so that the energy changes occurring in the course of the reaction produce a "good" peak; hence, again, the use of higher rates of heating in differential thermal analysis. For quantitative thermogravimetry, however (particularly if evaluation of kinetic parameters of the reaction is to be attempted), both of these operations would not be considered good practice. Garn[173] has discussed various aspects of this problem. An apparatus, such as that described by Hodgson,[107] offers a compromise, in that both procedures are available to the operator but as completely independent operations. The use of the two procedures permits information to be obtained on all phenomena listed on p. 906. Examination of a material by both procedures permits crystalline transitions, second-order transitions and solid - solid reactions occurring without weight change to be distinguished from a typical decomposition reaction, or indeed any of the phenomena listed on p. 906 that give rise to a weight change.

OTHER PROCEDURES—

In many of the studies discussed, one of the main uses of thermogravimetry has been the investigation of the thermal stability of the material,[13,149,322] or to find a suitable drying temperature for the investigation of a weighing form of an element.[143 to 146] However by using thermogravimetry together with infrared studies, X-ray diffraction[21] or electron-diffraction studies, or magnetic measurements—say, by heating up to a suitable point on the thermogravimetric curve (up to a point where a reaction has occurred) and removing the sample for examination by one or more of these methods—information can be gained on what structural changes have occurred during the course of the reaction. Thus a combination of infrared studies and magnetic measurements with a detailed thermogravimetric examination of the dehydration of hexa-aquochromium^{III} phosphate and arsenate enabled Lukaszewski and Redfern[58,166] to propose a reaction scheme for the dehydration reactions. A thermogravimetric study, coupled with X-ray diffraction studies and a study of catalytic activity,[7] of a hydrated ruthenium dioxide provided preliminary information on some structural changes occurring on heating.

CONCLUSION

It is evident from the volume of literature now appearing[1] that both thermogravimetric and differential thermal analysis have found many varied applications both in analytical chemistry and in other fields. It is likely that the introduction of more sophisticated instrumentation (with facilities for operating in inert, oxidising or otherwise controlled atmospheres) will lead to further developments, particularly in the fields of organic and inorganic materials and to the use of thermogravimetry directly as an analytical method. It is to be hoped that prospective workers in the field will realise the dynamic nature of the method and of its dependence on the factors outlined in the section on "Techniques."

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Analytical Methods Committee

REPORT OF THE ADDITIVES IN ANIMAL FEEDING STUFFS SUB-COMMITTEE

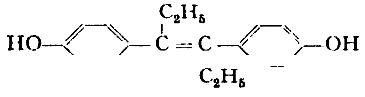
PART 2.* REPORT OF THE HORMONES PANEL

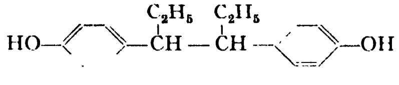
The Determination of Stilboestrol and Hexoestrol in Compound Feeding Stuffs

GENERAL INTRODUCTION

THE Hormones Panel was set up under the chairmanship of Dr. R. E. Stuckey, and its membership was: Mr. J. Allen, Mr. L. Brealey, Mr. J. A. Potter and Mr. W. L. Sheppard, with Miss A. M. Parry as Secretary. The Panel was appointed to consider methods for determining synthetic hormones included in animal rations as stimulants.

A number of synthetic hormones, both with an anabolic and an oestrogenic action, have been tried in practice as additives in animal feeding stuffs; they are stilboestrol, hexoestrol, dienoestrol, dianisyl hexane, dianisyl hexene, dianisyl hexadiene and dienoestrol acetate. However, only two—stilboestrol and hexoestrol—were considered by the Panel to be sufficiently important to warrant the detailed investigation necessary; their structures are shown below—


Stilboestrol


Hexoestrol

Although both these compounds possess phenolic groupings and can be assayed, when present in small amounts, by the use of a reagent that reacts with the phenol group, it was considered that some means of differentiating between the two compounds when present in feeding stuffs would be desirable.

However, early in the consideration of the assay methods available for these compounds, it was apparent that the methods of choice for stilboestrol and hexoestrol would be different. In fact, although the two compounds are chemically similar, the methods recommended are quite distinct and will be described separately.

STILBOESTROL

Stilboestrol has been known for many years, and methods for its determination, both in pharmaceuticals and in biological materials, have appeared in the literature; most of the chemical methods are based on reactions involving the phenolic group. Methods proposed include an ultraviolet-absorption method,[1] Folin's colorimetric methods,[2] a bromimetric method,[3] a nitroso method,[4] the irradiation method[5] and the antimony pentachloride method.[6]

The main problem in the chemical assay of stilboestrol in feeding stuffs lies in the extraction of the stilboestrol in a sufficiently pure form. Cheng and Burroughs,[7] working on a pre-mix containing 500 mg of stilboestrol per lb of soyabean meal, and a low-potency cattle diet supplement containing 5 mg of stilboestrol per lb in the same meal, described methods for determining stilboestrol in feeds. In these methods the stilboestrol was isolated by chromatography on a column of Celite, and the purified stilboestrol determined by measurement of the red colour produced by reaction with antimony pentachloride in ethylene chloride; alternatively, the extracted stilboestrol was determined by a method involving ultraviolet irradiation of a solution in 80 per cent. acetic acid and measurement of the resulting colour at 410 mμ. It was found that the colour in both methods obeyed Beer's law over the range 5 to 30 μg of stilboestrol, although the authors reported that the irradiation method did not give consistent results on the diet supplements, and the results with the antimony pentachloride method were high, probably because interfering substances in the feeds were not completely removed by chromatography. For concentrations of stilboestrol less than 5 mg per lb, the authors preferred a biological assay—a mouse-uterine weight method.[8]

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* Part 1 (Report of the Antibiotics Panel) appeared in *The Analyst*, **1963, 88, 835.**

In 1956 Hedger and Manley,[9] after a study of several chemical assay methods, reported that they preferred the ultraviolet-irradiation method, and described a procedure with which they reported recoveries of 90 to 98 per cent. of known amounts of stilboestrol added to feeds. In 1958 Hanka and Lockhart[10] reported that stilboestrol exerted a marked and specific inhibitory effect on the growth of *Staphylococcus aureus* strain H, and that this effect could be used for determining stilboestrol microbiologically by broth or disc-plate techniques.

The Panel finally decided that a chemical assay would be more generally useful than a microbiological or biological method. The irradiation method was in regular use by one of the members of the Panel, and it was decided that this method, modified from that of Hedger and Manley, should first be tried.

EXPERIMENTAL AND RESULTS

The steps in the procedure used were—

(*i*) mixing of the feed, grinding if necessary, and sampling for analysis;

(*ii*) extraction of stilboestrol from the sample with chloroform;

(*iii*) washing of the chloroform extract with N sulphuric acid to remove impurities;

(*iv*) extraction of the purified chloroform extract with sodium hydroxide solution;

(*v*) adjustment of the sodium hydroxide solution to pH 9·0 to 9·5 with 2 N orthophosphoric acid, and re-extraction of the stilboestrol with chloroform;

(*vi*) evaporation of the purified chloroform extract to dryness, dissolution of the residue in glacial acetic acid, and irradiation of the solution with ultraviolet light;

(*vii*) measurement of the optical density at 420 mμ and calculation of the stilboestrol present in the original feed by reference to a calibration graph.

Collaborative tests by the method outlined above were first carried out on a sample of soyabean meal to which 11 p.p.m. (about 5 mg per lb) of stilboestrol had been added.

In the first instance the Panel members who had not previously used the method experienced trouble at the irradiation stage. It was found that the wavelength of the light used to irradiate the solutions was critical, the shorter wavelength of 254 mμ being satisfactory, whereas the colour of the solution was destroyed by radiation of wavelength 365 mμ. It was also found essential to adhere closely to the distances and times specified in the method. After this had been realised and the correct lamp used, no difficulty was experienced in developing the maximum colour. The first results obtained are shown in Table I.

TABLE I

DETERMINATION OF STILBOESTROL IN SOYABEAN MEAL

Sample contained 11 p.p.m. of stilboestrol

Laboratory	Stilboestrol found, p.p.m.
A	8·8, 11·0
B	8·0
C	9·0
D	—

As an additional difficulty some samples of glacial acetic acid were found to be unsatisfactory, even when the procedure at the colour-development stage was carried out exactly as described in the method. Examination and fractionation of the acid suggested the presence, in some batches, of an inhibiting substance that was extremely difficult to remove. Careful fractionation sometimes produced a satisfactory product, but this did not always remain so on storage. Satisfactory samples of glacial acetic acid were circulated for use by the Panel members, and at this stage it was decided that a special test to assess the suitability of the acid used would have to be included in the reagent specification in the final method.

A further collaborative test was carried out on another sample of compound feeding stuff to which 10 p.p.m. of stilboestrol had been added. Considerable difficulty was again experienced in preparing glacial acetic acid satisfactory for the development of the colour by irradiation. At this stage, however, the United States Pharmacopoeia XVI was published, and it was noted that, in the official assay for stilboestrol,[11] phosphate buffer solution instead of glacial acetic acid was used as a solvent at the irradiation stage. A trial of this buffer solution showed that it was much preferable to glacial acetic acid and subject to none of its disadvantages. It was therefore agreed that this solvent should be substituted for glacial acetic acid at the colour-development stage. The results obtained in the collaborative test with the revised method are shown in Table II.

TABLE II

DETERMINATION OF STILBOESTROL IN A COMPOUND FEEDING STUFF

Sample contained 10 p.p.m. of stilboestrol

Laboratory	Stilboestrol found, p.p.m.	Remarks
A	(*a*) 8·1 8·9	Glacial acetic acid used
	(*b*) 8·8 7·3	Phosphate buffer solution used
	(*c*) 8·8	Phosphate buffer solution used on same extract as in (*a*)
B	9·4 9·7	Phosphate buffer solution used (colour developed more quickly)
C	(*a*) 8·4 9·1	Glacial acetic acid used (with a Hanovia lamp the colour was destroyed if the optimum time was exceeded)
	(*b*) 8·8	Phosphate buffer solution used
D	8·4	Phosphate buffer solution used

In view of the general agreement between the results and the possibility of incomplete incorporation of all the stilboestrol added during the preparation of the compound feeding stuff, it was considered by the Panel that this method was satisfactory and could be recommended; the method is described in detail in Appendix I.

HEXOESTROL

The determination of hexoestrol in pharmaceuticals and in biological materials has been described in the literature. The most common method and that described in the British Pharmaceutical Codex 1959 for determining hexoestrol in tablets depends on the measurement of the blue colour developed with sodium molybdophosphotungstate.

The use of hexoestrol in compound feeding stuffs is not as widespread as that of stilboestrol, and methods for determining hexoestrol specifically in feeding stuffs have not been described in the literature. Measurement of the colour developed with sodium molybdophosphotungstate is a sensitive method for determining hexoesterol, but the difficulty in applying it to feeding stuffs lies in isolating the hexoestrol in a sufficiently pure form.

After consideration of the methods available, the Panel decided to rely on the experience of one of its members, whose laboratory had devised a method for the chromatographic separation of hexoestrol and its subsequent purification.

EXPERIMENTAL AND RESULTS

The steps in the method initially tried by the Panel, but which was subsequently modified, were—

(*i*) mixing of the feed, grinding, sifting, and sampling for analysis;
(*ii*) extraction of hexoestrol from the sample with chloroform;
(*iii*) washing of the chloroform extract with N sulphuric acid to remove impurities;
(*iv*) extraction of the purified chloroform extract with sodium hydroxide solution;
(*v*) adjustment of the sodium hydroxide solution to pH 9·0 to 9·5 with 2 N orthophosphoric acid, and re-extraction of the hexoestrol with chloroform;
(*vi*) evaporation of a portion of the chloroform extract to dryness, and dissolution of the residue in a mixture of tetrahydrofuran, triethylamine and water;

(*vii*) removal of phenolic impurities by reversed-phase chromatography on a column of oleated cellulose powder;

(*viii*) elution of the hexoestrol from the cellulose with ether, and then extraction of the solution with sodium hydroxide solution;

(*ix*) acidification of the sodium hydroxide solution and re-extraction of the hexoestrol with ether;

(*x*) evaporation of the filtered ethereal extract, reaction with sodium molybdophosphotungstate reagent, measurement of the optical density of the coloured complex at 750 mμ and determination of hexoestrol by comparison with standards.

Collaborative tests were carried out by the method outlined above on a sample of soyabean meal to which 11 p.p.m. (about 5 mg per lb) of hexoestrol had been added.

In the first test, difficulties with the method were reported; the results obtained are shown in Table III.

TABLE III

DETERMINATION OF HEXOESTROL IN SOYABEAN MEAL

Sample contained 11 p.p.m. of hexoestrol

Laboratory	Hexoestrol found, p.p.m.
A	12·0, 10·4
B	7·2
C	8·8
D	—

Difficulties were experienced owing to the formation of emulsions during the sodium hydroxide - chloroform extraction stage and also to the fact that the final solutions for the colour measurement were often cloudy to different degrees. Incomplete purification, it was found, could also cause high results owing to the presence of extracted substances that reacted with the sodium molybdophosphotungstate.

It was decided that, at some stages, the assay procedure ought to be more precisely described, particularly at the stages relating to the extraction and the chromatography, and that the reagent grades ought to be specified.

A second collaborative test was carried out on a further sample of cattle feeding stuff to which 10 p.p.m. of hexoestrol had been added. During this second test one laborotory reported high results (about 15 p.p.m.), and it was suspected that fatty material from the chromatographic column might be responsible; this interference was subsequently minimised by describing in detail each step of the purification stage, by specifying a purified oil for the preparation of the oleated cellulose, and by removing any cloudiness in the final extract by filtration through sand. High results were also attributed to impurities in the solvent, but these could be avoided by distilling the ether before use. Other members reported occasional high results, which, it was considered, would be avoided if the ether were freshly distilled. Further, it was considered that any cloudiness occurring in the final extract should be removed by filtration through sand. The results obtained by the collaborating laboratories in a final collaborative test with the method described in Appendix II are shown in Table IV.

TABLE IV

DETERMINATION OF HEXOESTROL IN A COMPOUND FEEDING STUFF

Sample contained 10 p.p.m. of hexoestrol

Laboratory	Hexoestrol found, p.p.m.
A	8·3, 8·9, 8·6, 8·5
B	9·0
C	9·3, 9·2
D	10·9

The results were considered to indicate that the method was satisfactory for determining hexoestrol in compound feeding stuffs, and could be recommended (see Appendix II).

Appendix I

RECOMMENDED METHOD FOR DETERMINING STILBOESTROL IN COMPOUND FEEDING STUFFS

PRINCIPLE OF METHOD—

The stilboestrol is extracted from the feeding stuff with chloroform, then taken into sodium hydroxide solution, and, after the solution has been adjusted to pH 9·0 to 9·5 with orthophosphoric acid, is re-extracted with chloroform. The purified chloroform extract is evaporated, and the residue dissolved in phosphate buffer solution. The optical density is measured at 418 mμ before and after irradiation of the solution with ultraviolet light, and from the difference the amount of stilboestrol in the sample is calculated by reference to the optical-density difference when an extract to which a known amount of stilboestrol has been added is similarly treated.

APPLICABILITY—

The method is suitable for the analysis of feeding stuffs containing about 11 p.p.m. (about 5 mg per lb) stilboestrol.

SPECIAL APPARATUS—

Extraction apparatus—Any apparatus may be used provided it permits (*i*) the uniform percolation of the powdered feeding stuff with the extracting solvent and (*ii*) the regular flow of the solvent vapour around the percolator. A suitable form consists of a glass tube, about 4 cm in internal diameter and 15 cm in length, with a piece of coarse filter-paper covered with fine calico firmly tied over the lower flanged end. This percolator rests on a glass spiral inside an outer tube, about 6 cm in internal diameter and about 25 cm in length, the spiral being supported on the shoulder of a B24 standard joint sealed to the lower end of the outer tube. This standard joint fits into a flask of suitable size to contain the solvent, and a reflux condenser is attached to the top of the tube. An alundum crucible of medium porosity and suitable size also forms a satisfactory percolator.

Irradiation equipment—This consists of a mercury-discharge tube mounted horizontally in a suitable reflector, together with a holder capable of containing four silica cells, so arranged that the cells are perpendicular to the light source and fixed at 15 cm (6 inches) from it. The holder is also positioned so that a line through the liquid centres in the cells is level with the centre of the discharged tube, and the cells themselves, spaced about 1 cm apart, are centrally located relative to the ends of the discharge tube.

The Phillips Germicidal tube TUV (15 watt), together with a trough fitting, A7003, with the grill removed, is a suitable source of radiation.

REAGENTS—

Washed sand.

Chloroform—Analytical-reagent grade. The chloroform used must comply with the test described below.

Shake 35 ml of the chloroform with 70 ml of water, and then allow the layers to separate. To 10 ml of the aqueous extract add 40 ml of distilled water and 2 ml of Nessler's reagent, and set aside in the dark for 15 minutes. No colour or turbidity should be produced.

Sulphuric acid, N.

Sodium hydroxide, N.

Orthophosphoric acid, 2 N.

Sodium sulphate, anhydrous.

Ethanol, absolute.

Potassium phosphate solution—A 1·8 per cent. w/v solution of dipotassium hydrogen orthophosphate, K_2HPO_4, in distilled water.

Stock standard stilboestrol solution—Prepare a solution in chloroform to contain exactly 0·55 mg of stilboestrol B.P. per ml.

Working standard stilboestrol solution—Dilute 10 ml of stock standard stilboestrol solution to 100 ml at 20° C with chloroform;

$$1 \text{ ml} \equiv 55 \ \mu\text{g of stilboestrol.}$$

PROCEDURE

EXTRACTION OF SAMPLE—

Crush about 1 kg of the sample, mix and grind 100 g of the crushed material until not less than 95 per cent. passes a 30-mesh sieve. Weigh accurately about 40 g of the ground material, and mix with about 10 g of washed sand. Transfer the mixture to a stoppered flask, add about 100 ml of chloroform, shake vigorously, and set aside overnight. Transfer the contents of the flask to the percolator of the extraction apparatus, collecting the chloroform in the flask, and assemble the apparatus. Extract the solid material for 6 hours; use more chloroform if necessary. Filter the extract through a pad of cotton-wool into a 200-ml calibrated flask, washing the extraction flask and filter with chloroform, and finally dilute the combined extract and washings to 200 ml with chloroform.

PURIFICATION OF EXTRACT—

Transfer a 25-ml portion of the solution from the extraction of sample, together with 25 ml of chloroform, to each of two separators; add exactly 1 ml of working standard stilboestrol solution to the contents of one separator, and treat the contents of each separator as described below.

Add 25 ml of N sulphuric acid, swirl gently for 30 seconds, avoiding as far as possible the formation of emulsions, allow the layers to separate for 10 minutes, and transfer the lower chloroform layer to another separator. If emulsions form at this stage, it may be helpful to spin the mixture in a centrifuge. Add 10 ml of chloroform to the aqueous acid layer, shake gently for 10 seconds, still taking care to avoid emulsification, allow the layers to separate and add the lower chloroform layer to the initial chloroform solution. Repeat this last operation with two further successive 10-ml portions of chloroform, shaking vigorously, and then discard the aqueous acid liquid.

Shake the combined chloroform solutions carefully for 30 seconds with two successive 10-ml portions of N sodium hydroxide; set aside for 10 minutes each time before running off the lower chloroform layer. Discard the chloroform solution. Combine the sodium hydroxide extracts, add 5 ml of chloroform, shake for 5 seconds, allow the layers to separate, and transfer the lower chloroform layer to the empty separator that previously held a sodium hydroxide extract. Repeat the extraction of the combined sodium hydroxide extracts with two or three successive 5-ml portions of chloroform until a chloroform layer is obtained that, after having been shaken and allowed to separate, is colourless; add each chloroform layer to the first one. Add 5 ml of distilled water to the combined chloroform washings, shake, allow the layers to separate, and run off and discard the chloroform layer. Transfer the washed alkaline solution and the aqueous washings to a 50-ml beaker; rinse the empty separator with successive small portions of distilled water until the washings are free from all alkalinity, and add the rinsings to the contents of the beaker.

To the combined alkaline aqueous solution and washings, add 4 ml of 2 N phosphoric acid, and adjust the solution carefully to pH 9·0 to 9·5 with 2 N phosphoric acid; use a pH meter to make the measurements. Return the adjusted solution to the same separator, rinsing the beaker first with two successive 2-ml portions of distilled water and then with 15 ml of chloroform, and add the rinsings to the contents of the separator. Swirl the mixture carefully for 30 seconds taking care to avoid the formation of an emulsion, allow the layers to separate, and transfer the lower chloroform layer to another separator. Add to the chloroform extract 25 ml of distilled water, shake for 5 seconds, allow the layers to separate, and filter the lower chloroform layer through a 1-inch bed of anhydrous sodium sulphate in a sintered-glass funnel; collect the filtrate in a 50-ml calibrated flask. Repeat the extraction of the alkaline aqueous solution with two successive 15-ml portions of chloroform; wash both the chloroform extracts with the same 25 ml of water, and then filter the washed extracts through sodium sulphate as described above. Shake the aqueous washings with successive small portions of chloroform; use these chloroform extracts to wash the sodium sulphate and the funnel, and then add to the contents of the calibrated flask. Continue this process until the solution in the flask is adjusted to the mark, and mix.

DETERMINATION OF STILBOESTROL—

Transfer a 25-ml portion of the sample solution from the purification of extract to a 100-ml beaker previously rinsed with chloroform, and evaporate off the solvent with a gentle current of air, warming the beaker on a water-bath until only the last traces of solvent remain. Dissolve the residue in 5 ml of absolute ethanol, warming gently, if necessary, to effect dissolution, and add 5 ml of potassium phosphate solution.

Measure the optical density at 418 mμ of the solution against water in a 1-cm cell with a spectrophotometer.

Transfer about 3 ml of the aqueous ethanolic solution to a silica cell, and irradiate with ultraviolet light for 10 minutes. Measure the optical density at 418 mμ of the irradiated solution against water in a 1-cm cell with a spectrophotometer. Re-irradiate the solution for successive 1-minute periods until the maximum optical-density reading is obtained. Repeat the irradiation on a further 3-ml portion of the aqueous ethanolic solution, irradiating for the period found previously to give the maximum optical density, and use this figure in the calculation.

Treat, in exactly the same manner, a 25-ml portion of the solution from the purification of extract obtained from the extract to which a known amount of stilboestrol has been added.

CALCULATION—

Calculate the amount of stilboestrol present in the sample from the expression—

$$\frac{A - a}{(B - b) - (A - a)} \times \frac{55}{2} \times \frac{8}{\text{Weight of sample}} \text{ p.p.m.,}$$

where A = optical density of sample solution after irradiation,
a = optical density of sample solution before irradiation,
B = optical density of sample solution with added stilboestrol after irradiation and
b = optical density of sample solution with added stilboestrol before irradiation.

Appendix II

RECOMMENDED METHOD FOR DETERMINING HEXOESTROL IN COMPOUND FEEDING STUFFS

PRINCIPLE OF METHOD—

The hexoestrol is extracted from the feeding stuff with chloroform, then taken into sodium hydroxide solution, and, after the solution has been adjusted to pH 9·0 to 9·5 with orthophosphoric acid, is re-extracted with chloroform. The purified chloroform extract is evaporated, the residue dissolved in an aqueous solution of tetrahydrofuran and triethylamine, and the hexoestrol separated by column chromatography. The hexoestrol is extracted with ether, the ethereal solution evaporated, the residue treated with a molybdophosphotungstic acid reagent, and the hexoestrol determined absorptiometrically.

APPLICABILITY—

The method is suitable for the analysis of feeding stuffs containing 10 p.p.m. (about 5 mg per lb) of hexoestrol.

SPECIAL APPARATUS—

Extraction apparatus—As described in the method for stilboestrol (see Appendix I, p. 929).

Chromatographic tube—A glass tube, about 1 cm in internal diameter and about 15 cm in length, drawn out at one end for a length of about 2 cm to terminate in a jet, about 3 mm in internal diameter, is suitable. In such a tube the adsorbent can be supported on a pledget of cotton-wool.

REAGENTS—

Washed sand.
Fine sand.
Cellulose powder.
Ether, redistilled—Anaesthetic ether B.P., freshly distilled.
Arachis oil solution—A 5 per cent. v/v solution of arachis oil B.P., in redistilled ether.
Chloroform—Analytical-reagent grade.
Sulphuric acid, N.
Sodium hydroxide solution, N.

Orthophosphoric acid, 2 N.

Sodium sulphate, anhydrous.

Tetrahydrofuran - triethylamine solution—A mixture of 5 volumes of triethylamine, 25 volumes of tetrahydrofuran and 70 volumes of distilled water.

Sulphuric acid, diluted—Mix carefully 1 volume of sulphuric acid, sp.gr. 1·83, with 9 volumes of distilled water.

Ethanol, 95 *per cent. v/v.*

Ethanol, diluted—Mix equal volumes of ethanol and distilled water.

Hydrochloric acid, diluted—Mix 1 volume of hydrochloric acid, sp.gr. 1·16 to 1·18, with 9 volumes of distilled water.

Molybdophosphotungstate reagent—Add 50 g of sodium tungstate, $NaWO_4.2H_2O$, 12 g of molybdophosphoric acid, $H_3PO_4.12MoO_3.24H_2O$, and 25 ml of orthophosphoric acid (88 per cent. w/w) to about 350 ml of distilled water in a round-bottomed flask. Boil the mixture under reflux for 2 hours, cool, and dilute to 500 ml with distilled water. Store in a well-stoppered bottle protected from light.

Sodium carbonate solution—A 10 per cent. w/v solution in distilled water.

Stock standard hexoestrol solution—Dissolve 50·0 mg of hexoestrol in diluted ethanol, and make up to 100 ml at 20° C with diluted ethanol.

Working standard hexoestrol solution—Dilute 5·0 ml of stock standard hexoestrol solution to 50 ml at 20° C with diluted ethanol;

1 ml ≡ 50 μg of hexoestrol.

Procedure

Extraction of sample—

Crush about 1 kg of the sample, mix, and grind about 100 g of the crushed material until not less than 95 per cent. passes a 30-mesh sieve. Weigh accurately about 40 g of the ground material, and mix with about 10 g of washed sand. Transfer the mixture to a stoppered flask, add about 100 ml of chloroform, shake vigorously, and set aside overnight. Transfer the contents of the flask to the percolator of the extraction apparatus, collecting the chloroform in the flask, and assemble the apparatus. Extract the solid material for 6 hours; use more chloroform if necessary. Filter the extract through a pad of cotton-wool into a 200-ml calibrated flask, washing the extraction flask and filter with chloroform, and finally dilute the combined extract and washings to 200 ml with chloroform.

Purification of extract—

Transfer a 50-ml portion of the solution from the extraction of sample to a separator, add 25 ml of N sulphuric acid, swirl gently for 30 seconds, avoiding as far as possible the formation of emulsions, allow the layers to separate for 10 minutes, and transfer the lower chloroform layer to another separator. If emulsions form at this stage, it may be helpful to spin the mixture in a centrifuge. Add 10 ml of chloroform to the aqueous acid layer, shake gently for 10 seconds, still taking care to avoid emulsification, allow the layers to separate, and add the lower chloroform layer to the initial chloroform solution. Repeat this last operation with two further successive 10-ml portions of the chloroform, shaking vigorously, and then discard the aqueous acid liquid.

Shake the combined chloroform solutions carefully for 30 seconds with two successive 10-ml portions of N sodium hydroxide; set aside for 10 minutes each time before running off the lower chloroform layer. Discard the chloroform solution. Combine the sodium hydroxide extracts, add 5 ml of chloroform, shake for 5 seconds, allow the layers to separate, and transfer the lower chloroform layer to the empty separator that previously held a sodium hydroxide extract. Repeat the extraction of the combined sodium hydroxide extracts with two or three successive 5-ml portion of chloroform until a chloroform layer is obtained that, after having been shaken and allowed to separate, is colourless; add each chloroform layer to the first one. Add 5 ml of distilled water to the combined chloroform washings, shake, allow the layers to separate, and run off and discard the chloroform layer. Transfer the washed alkaline solution and the aqueous washings to a 50-ml beaker; rinse the empty separator with successive small portions of distilled water until the washings are free from all alkalinity, and add the rinsings to the contents of the beaker.

To the combined alkaline aqueous solution and washings add 4 ml of 2 N phosphoric acid, and adjust the solution carefully to pH 9·0 to 9·5 with 2 N phosphoric acid; use a pH meter to make the measurements. Return the adjusted solution to the same separator, rinsing the beaker first with two successive 2-ml portions of distilled water and then with 15 ml of chloroform, and add the rinsings to the contents of the separator. Swirl the mixture carefully for 30 seconds, taking care to avoid the formation of an emulsion, allow the layers to separate, and transfer the lower chloroform layer to another separator. Add to the chloroform extract 25 ml of distilled water, shake for 5 seconds, allow the layers to separate and filter the lower chloroform layer through a 1-inch bed of anhydrous sodium sulphate in a sintered-glass funnel; collect the filtrate in a 50-ml calibrated flask. Repeat the extraction of the alkaline aqueous solution with two successive 15-ml portions of chloroform; wash both chloroform extracts with the same 25 ml of water, and then filter the washed extracts through sodium sulphate as described above. Shake the aqueous washings with successive small portions of chloroform; use these chloroform extracts to wash the sodium sulphate and the funnel, and then add them to the contents of the calibrated flask. Continue this process until the solution in the flask is adjusted to the mark, and mix.

ISOLATION AND DETERMINATION OF HEXOESTROL—

Preparation of adsorption column—Mix 2 g of cellulose powder with 6 ml of arachis oil solution, add more ether, and mix thoroughly, evaporating off the ether during the mixing. Make the residual oleated cellulose into a thin slurry with tetrahydrofuran - triethylamine solution, transfer to the chromatographic tube, and allow to drain.

Treatment of test solution—Transfer a 25-ml portion of the chloroform solution from the purification of the extract to a 100-ml beaker, and evaporate off the solvent with a gentle current of air, warming the beaker gently on a water-bath until only the last traces of solvent remain. Dissolve the residue in the minimum amount of tetrahydrofuran - triethylamine solution, and transfer the solution to the top of the prepared adsorption column. Allow the solution to percolate into the column until the surface of the liquid just disappears below the surface of the cellulose, stopper the upper end of the tube, and set aside for 1 hour. Transfer 10 ml of tetrahydrofuran - triethylamine solution to the top of the adsorption column, and allow the liquid to run through the column to remove impurities. When the flow has ceased, remove the residual liquid from the column by applying gentle suction at the bottom of the tube.

Transfer the cellulose column from the tube to a separator, wash out the tube with a little ether, and add the washings to the contents of the separator. Add 1 ml of N sulphuric acid and 20 ml of ether, shake, allow the layers to separate, and transfer the upper ethereal layer to another separator. Repeat the extraction of the acidified cellulose suspension with two successive 20-ml portions of ether, adding the ethereal layers to the ethereal extract in the second separator. To the combined ethereal extracts, add 10 ml of N sodium hydroxide, shake, and allow the layers to separate. Transfer the lower aqueous layer to another separator containing 5 ml of ether, shake, allow the layers to separate and transfer the lower aqueous layer to a third separator. Repeat the extraction of the combined ethereal extracts, first with a further 10-ml portion, and then with three successive 5-ml portions, of N sodium hydroxide; wash each aqueous alkaline extract with the same 5 ml of ether, and discard the ethereal solution and washings. Combine the washed aqueous alkaline extracts, acidify with diluted sulphuric acid, add 10 ml of ether, shake, and allow the layers to separate. Transfer the upper ethereal layer to another separator containing 5 ml of distilled water, shake, allow the layers to separate and transfer the upper ethereal layer to a 50-ml flask. Repeat the extraction of the acidified aqueous extracts, first with a further 10-ml portion, and then with three successive 5-ml portions, of ether; wash each ethereal extract with the same 5 ml of water before it is added to the first extract in the flask. Filter the combined ethereal extracts through a 1-cm layer of fine sand, wash the filter with several small portions of ether and evaporate the combined filtrate and washings to a volume of about 5 ml by gentle warming. Transfer the concentrated solution quantitatively to a 14-ml calibrated centrifuge tube with the aid of more ether, and carefully evaporate the ethereal solution to dryness.

Dissolve the residue in 0·5 ml of ethanol, add 0·5 ml of distilled water and then 0·4 ml of diluted hydrochloric acid, 0·8 ml of molybdophosphotungstate reagent and 5 ml of distilled water, mix well, and set aside for 10 minutes. Add 3 ml of sodium carbonate solution and sufficient distilled water to produce 12 ml, mix, set aside for 1 hour, and spin in a centrifuge for 15 to 20 minutes. Measure the optical density at 750 mμ of the clear supernatant liquid with a spectrophotometer in a 1-cm cell against a blank solution prepared by adding 0·4 ml of dilute hydrochloric acid, 0·8 ml of molybdophosphotungstate reagent and 5 ml of water to 1 ml of diluted ethanol, and continue as described above, commencing at the words "mix well, and set aside for 10 minutes . . ."

Calculate the amount of hexoestrol in the solution by comparison of the optical density with that obtained by treating 0·8, 1·0 and 1·2 ml of working standard hexoestrol solution in the same manner, beginning with the addition of 0·4 ml of dilute hydrochloric acid in the previous paragraph; the optical densities of the test solution and of the chosen standard solution should not differ by more than 10 per cent. Hence calculate the amount of hexoestrol in the sample.

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PART 3. REPORT OF THE PROPHYLACTICS PANEL

The Determination of Nitrofurazone in Compound Feeding Stuffs

INTRODUCTION

THE Prophylactics Panel was set up under the chairmanship of Dr. R. F. Phipers, and its membership was: Mr. C. W. Ballard, Mr. N. C. Brown, Dr. H. G. Dickenson, Mr. A. W. Hartley, Mr. A. Holbrook, and Mr. S. G. E. Stevens, with Miss A. M. Parry as Secretary; Mr. J. A. Stubbles and Mr. D. H. Mitchell were co-opted later. The Panel was appointed to consider methods of analysis for determining medicaments against coccidiosis and histomoniasis included in poultry rations.

The Panel turned its attention first to the determination of nitrofurazone, which is widely used as a poultry coccidiostat, and this report describes the results of collaborative tests that led to the recommended method being advanced.

There is a considerable volume of published information on the analysis of nitrofurazone in animal feeding stuffs, and it was not expected that there would be any difficulty in making a recommendation. However, the Panel's investigations showed that this was not so, and the method finally evolved contains various recommendations that, if ignored, will lead to inaccurate results being obtained.

Ells, McKay and Paul[1] described an extraction method for separating nitrofurazone from feeding stuffs, and they compared the optical densities of such extracts with the optical densities of similar solutions in which the nitrofurazone had been reduced by treatment with aqueous sodium dithionite solution. In recent years the complexity of the components used in the formulation of poultry feeding stuffs has increased; the introduction of grass meals and other vegetable materials yielding highly coloured extracts has rendered valueless simple methods of extraction and reduction. Buzard, Ells and Paul[2] attempted to overcome these difficulties by allowing the nitrofurazone extracted from medicated feeding stuffs to react with phenylhydrazine hydrochloride and measuring the red colour produced. Collaborative trials recorded by Puglisi[3] showed that this procedure was unreliable. van Zijl and Goosens[4] claimed that they could overcome interference from reducing and other materials, by oxidation of the sample solution with potassium permanganate. Tagaki and Uno[5] found that the addition of caustic alkali to nitrofurazone solutions led to an intensification of the colour, but the orange-red colour thus produced is unstable, and any determinations involving its use are difficult.

Cross, Hendey and Stevens[6] took advantage of some investigations by Porter,[7] who studied the colour reactions of certain nitro-compounds, and they adopted his use of dimethylformamide as a solvent. They found that the addition of alkali to solutions of nitrofurazone in dimethylformamide intensified the colour; this was of obvious value in spectrophotometric work, but they noted that the colour of the resulting solution required stabilisation before it could be used on a quantitative basis. Phenol was found to confer the desired degree of stability.

Cross, Hendey and Stevens[6] extracted a typical medicated poultry feeding stuff, *i.e.*, one containing about 0·01 per cent. of nitrofurazone, with light petroleum in a Soxhlet apparatus. Nitrofurazone is insoluble in light petroleum, and this first step permitted much interfering material to be removed. This extraction was followed by a similar treatment with carbon tetrachloride, in which solvent nitrofurazone also is essentially insoluble. The nitrofurazone was then extracted with acetone from the pre-extracted meal in a Soxhlet apparatus. After removal of acetone from the extract, the residue was dissolved in dimethylformamide. Suitable amounts of phenol, dissolved in dimethylformamide, and then aqueous alkali, were added. Two portions of the solution were taken, and one was treated with sodium dithionite. After centrifugation, the optical density of each solution was measured, and from the difference the amount of nitrofurazone present was obtained by reference to a calibration graph prepared by similarly treating known amounts of nitrofurazone.

EXPERIMENTAL AND RESULTS

Information on the above method was given in a personal communication from Mr. S. G. E. Stevens early in the development of the method before the use of sodium dithionite had been introduced. A collaborative test with this early version of the method was carried out by members of the Panel on feeding stuffs of differing complexity. It will be seen from Table I, which gives the results obtained on meals of increasing complexity, that satisfactory results were obtained on the simple Meal A, but on the more complex meals, it is clear that the pre-extraction procedure failed to remove interfering materials.

TABLE I

EFFECT OF ADDITION OF GRASS MEAL ON THE RECOVERY OF NITROFURAZONE FROM A FEEDING STUFF

Type of feed	Nitrofurazone present in sample, %	Recovery, %
Meal A	0·100	94
Meal A + 10 per cent. of grass meal ..	0·100	95
Meal B	0·010	110
Meal B + 10 per cent. of grass meal ..	0·010	135
Meal C	0·005	118
Meal C + 10 per cent. of grass meal ..	0·005	144

In subsequent assays the use of sodium dithionite to provide an empirical blank solution gave somewhat lower results more closely approximating to the theoretical values. It became apparent that, with some complex feeding stuffs, the extractives contained substances having reducing properties. This was demonstrated by measuring, at intervals of time, the optical density of the colour produced from an extract from a complex medicated meal containing nitrofurazone; the value decreased progressively.

The Panel then examined the use of potassium permanganate as described by van Zijl and Goosens,[4] and a modification of the method, in which the permanganate was added drop by drop, yielded the results shown in Table II.

TABLE II

EFFECT OF TREATMENT WITH POTASSIUM PERMANGANATE ON THE RECOVERY OF NITROFURAZONE FROM A COMPLEX FEEDING STUFF

Type of feed	Laboratory	Nitrofurazone added, %	Recovery (mean of 3 results), %
Without grass meal ..	A	0·005	99
		0·010	98
	B	0·005	78
		0·010	83
	C	0·005	93
		0·010	94
	D	0·005	94
		0·010	93
With grass meal ..	A	0·005	99
		0·010	98
	B	0·005	100
		0·010	92
	D	0·005	83
		0·010	90

During attempts to reconcile the discrepancies that continued to appear in the collaborative tests, it became evident that the quality of the dimethylformamide was important; the purity of this reagent should be such that the colour developed in the phenol and sodium hydroxide solutions in the presence of nitrofurazone should be stable for at least 2 hours. It was also established that the sodium dithionite solution must be freshly prepared and that the solid sodium dithionite itself should not be more than 6 months old. Some members also observed that if the meal were medicated by adding to it a portion of an acetone solution of the drug, low recoveries of the nitrofurazone were obtained. This point is illustrated in Table III.

TABLE III

EFFECT OF METHOD OF ADDING THE DRUG ON THE RECOVERY OF NITROFURAZONE

Laboratory	Nitrofurazone added, %	Recovery, %	Method of medication
A	0·005	97, 95	Solid drug added
	0·010	99, 102	
B	0·005	100, 97	Drug added in solution after extraction with carbon tetrachloride
	0·010	97, 100	
	0·010	52, 62 62	Drug added to feed as solution in acetone
C	0·005	96, 94	Solid drug added
	0·010	93, 97	
D	0·005	87, 86	Solid drug added
	0·010	87, 87	
	0·005	37, 33	Drug added as solution in acetone
	0·010	39, 39	
E	0·005	93	Solid drug added
	0·010	96, 95 99, 94 94	
	0·010	56, 57 62	Drug added as solution in acetone

Despite modifications to the experimental techniques, the Panel continued to obtain results lacking a reasonable degree of concordance. The factors detailed below were therefore examined.

(*i*) The suitability of dimethylformamide as a solvent in the development of colour.
(*ii*) The effect of time of centrifugation.
(*iii*) The effect of potassium permanganate on a solution of nitrofurazone in acetone.
(*iv*) The effect of potassium permanganate on extracts of feeding stuffs medicated with nitrofurazone.
(*v*) The extraction process.

Detailed investigation showed that the quality of the reagents and the extraction process were the only significant sources of variation.

A series of experiments demonstrated that the intensity of the colour produced was linearly proportional to the concentration of nitrofurazone in the range 0 to 0·0004 per cent. (Beer's law) and also that the calibration curves prepared by individual members of the Panel did not vary by more than ±3 per cent.

By careful definition of the procedure, the Panel members were able to obtain better agreement, although the recoveries were in the range of only 80 to 90 per cent. For medicated feeds produced in bulk, two explanations were considered: (*a*) electrostatic deposition of the nitrofurazone in the mixing equipment and (*b*) the possibility of instability of the nitrofurazone in the feeding stuff. Experiments showed that the first of these possible explanations was not true when adequate earthing precautions had been taken. It was still not clear whether the observed losses were due to degradation of nitrofurazone in the feeding stuff itself or during the pre-treatment of the sample. It was shown that satisfactory results were obtained on fairly old samples examined by members of the Panel when only light petroleum was used for the preliminary extraction. However, if carbon tetrachloride was used as the only pre-extraction solvent, low recoveries were sometimes obtained. Further, it was shown that if such feeding stuffs were extracted with carbon tetrachloride before medication with nitrofurazone then the use of carbon tetrachloride as a pre-extraction solvent caused no losses. These findings appear to indicate that there is some interaction between the nitrofurazone and the materials either extracted from the feed or derived from carbon tetrachloride; possibly decomposition of nitrofurazone may also result from prior decomposition of carbon tetrachloride itself as a result of contact with other constituents in the feed. The Panel has not conducted an investigation into this degradation; they consider that it is sufficient to draw attention to it.

Therefore the original method, in which only light petroleum is used as the pre-extraction solvent, is recommended, and any subsequent use of carbon tetrachloride will be necessary only when results obtained by this method indicate a complexity in the feeding stuff such as to cause undue interference with the optical measurements.

The foregoing remarks are only applicable to the analysis of the compound feeding stuffs now commonly marketed and containing only nitrofurazone as the coccidiostat. The analyst should acquaint himself with changes in the composition of the feeding stuff, and he should consider the implications arising from the introduction of new components.

RESULTS OF COLLABORATIVE TESTS—

Members of the Panel carried out collaborative tests by the recommended method (see Appendix I). Two materials were examined, the first of these being a compound feeding stuff containing 0·0060 per cent. of nitrofurazone, prepared by a member of the Panel

TABLE IV

RESULTS OBTAINED BY THE RECOMMENDED METHOD ON A MEDICATED POULTRY FEED

Sample contained 0·0060 per cent. of nitrofurazone

Laboratory and Analyst	Nitrofurazone found, %	Recovery, %	Laboratory and Analyst	Nitrofurazone found, %	Recovery, %
B1	0·0060	100	E1	0·0059	98
	0·0060	100		0·0065	108
	0·0060	100		0·0060	100
C1	0·00600	100		0·0059	98
	0·00605	101	E2	0·0056	93
	0·00595	100		0·0056	93
C2	0·00565	94		0·0054	90
	0·00570	95		0·0057	95
	0·00565	94	E3	0·0058	97
D1	0·0063	105		0·0059	98
	0·0063	105		0·0063	105
	0·0061	102		0·0059	98
D2	0·0059	98	F1	0·00625	104
	0·0057	95		0·00645	108
	0·0057	95		0·00620	103
E1	0·0057	95		0·00625	104
	0·0057	95		0·00605	101
	0·0053	88		0·00625	104

TABLE V

RESULTS OBTAINED BY THE RECOMMENDED METHOD ON MEDICATED POULTRY FEEDS

Samples prepared by adding different known amounts of nitrofurazone to an unmedicated poultry feed

Laboratory and Analyst	Nitrofurazone added, %	Nitrofurazone found, %	Recovery, %
B1	0·0080	0·00808	101
	0·0098	0·00941	96
	0·0100	0·01080	108
C1	0·0052	0·00535	103
	0·0060	0·00595	99
	0·0062	0·00620	100
C2	0·0055	0·00565	103
	0·0079	0·00815	103
	0·0087	0·00895	103
D1	0·0060	0·0060	100
	0·0074	0·0072	97
	0·0076	0·0073	96
D2	0·0073	0·0070	96
	0·0080	0·0073	91
	0·0089	0·0083	93
E1	0·00658	0·00660	100
	0·00700	0·00680	97
	0·00709	0·00700	99
	0·00712	0·00695	98

experienced in feed compounding. The second sample was prepared by each worker from the same unmedicated feeding stuff to which he added a known amount of nitrofurazone within the range 0·005 to 0·01 per cent. The results on these two samples are shown in Tables IV and V, respectively.

Appendix I

RECOMMENDED METHOD FOR DETERMINING NITROFURAZONE IN COMPOUND FEEDING STUFFS

PRINCIPLE OF METHOD—

The nitrofurazone is extracted by a selective solvent-extraction procedure, and then treated with alkali in the presence of phenol. The stabilised colour developed is compared with that produced by a known amount of the drug, similarly treated.

APPLICABILITY—

The method is applicable to medicated pre-mixes and compound feeding stuffs, of the type marketed at the time this Report was prepared, containing nitrofurazone as the only coccidiostat.

APPARATUS—

Soxhlet extractor—A 100-ml extractor fitted with a B34 socket and a B24 cone (B.S. 2071:1954).

Extraction thimble—Whatman single, 25 mm × 80 mm.

REAGENTS—

Light petroleum, boiling-range 40° *to* 60° *C.*

Acetone—Analytical-reagent grade.

Dimethylformamide—*N*,*N*-Dimethylformamide. Test the suitability of the reagent by developing the colour from nitrofurazone with solutions of phenol and sodium hydroxide (see "Procedure" below); the colour should remain stable for at least 2 hours.

Phenol solution—A 5 per cent. w/v solution in dimethylformamide.

Potassium permanganate solution, 0·1 N.

Sodium hydroxide solution, N.

Sodium dithionite solution—A 1 per cent. w/v solution of sodium dithionite, $Na_2S_2O_4$ (sodium hydrosulphite), (not more than 6 months old) in N sodium hydroxide. Prepare this solution immediately before use.

Nitrofurazone—Complying with the requirements of the British Veterinary Codex, 1953, p. 240.

PROCEDURE

PRELIMINARY EXTRACTION OF SAMPLE—

Weigh accurately an amount of sample containing about 1 mg of nitrofurazone, and transfer it to the extraction thimble; cover the sample with a small pad of cotton-wool. Insert the packed thimble into the extractor, assemble the extraction apparatus, and extract the sample with light petroleum; use an electric pad as the source of heat, so adjusted that the solvent cycles twenty times in about 45 minutes, and sufficient solvent so that the volume in the flask throughout the operation is not less than 25 ml.

Remove the packed thimble, allow the solvent to drain, and carefully remove any residual solvent in a current of warm air at a temperature not exceeding 60° C.

EXTRACTION OF NITROFURAZONE—

Transfer the packed thimble to a clean extraction apparatus, and extract the sample with acetone; use a water-bath as the source of heat so that the solvent cycles twenty times in about 1 hour, and sufficient solvent so that the volume in the flask throughout the operation is not less than 25 ml. During this extraction, shield the apparatus from light with a cardboard cylinder containing a small inspection window, or by any other suitable means.

When the extraction is complete, rapidly cool the flask containing the extract to 20° C, and add 0·1 N potassium permanganate, drop by drop, until a faint pink colour is obtained that is persistent for about 2 seconds (about 4 drops are required). Evaporate the extract on a water-bath to a volume of about 5 ml, shielding the extract from light. *It is important at this stage to avoid evaporating to dryness.*

Remove the flask from the water-bath, place an externally ribbed conical filter into the neck of the flask, and evaporate off the residual acetone by blowing a current of warm air (temperature not exceeding 60° C) across the top of the funnel in a way such that a slight turbulence is produced on the surface of the liquid in the flask.

DETERMINATION OF NITROFURAZONE—

Dissolve the residue in dimethylformamide, transfer the solution quantitatively to a 50-ml calibrated flask, suitably shielded from light, and dilute to the mark at 20° C with dimethylformamide. Transfer a suitable portion, containing about 0·3 mg of nitrofurazone, to each of two 50-ml calibrated flasks containing 5 ml of phenol solution.

To the contents of one flask add 2·5 ml of N sodium hydroxide, and dilute to the mark at 20° C with dimethylformamide; this is the *sample solution*. To the contents of the other flask add 2·5 ml of sodium dithionite solution, and dilute to the mark at 20° C with dimethylformamide; this is the *blank solution* and it should be a pale lemon-yellow colour free from any red or purplish tinge. Spin the solutions in a centrifuge, with a radius of 6 cm, at a speed of not less than 4000 r.p.m. for 2 minutes.

Measure the optical density at 530 mμ of the clear sample solution against the blank solution in 1-cm cells with a suitable spectrophotometer.

Obtain the amount of nitrofurazone present in the sample solution by reference to a calibration curve previously prepared by plotting the optical densities obtained when known amounts of nitrofurazone are treated as described above, beginning at the addition of a suitable portion to each of two 50-ml calibrated flasks containing 5 ml of phenol solution. Hence calculate the amount of nitrofurazone in the sample.

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Improvement of Identification in the Gas - Liquid Chromatographic Analysis of Agricultural Samples for Residues of some Chlorinated Pesticides*

Part I. Improvement of Resolution on Single Columns and Application of the Multi-column "Spectrochromatogram"

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Gas - liquid chromatography with detection by electron-capture ionisation has shown itself to be a technique of considerable value in the analysis of crops, soils and animal tissues for residues of the chlorinated pesticides. The chief limitation of the method, however, lies in incomplete certainty of identification of a pesticide based on retention time alone on a single column with the resolving power at present available.

Two methods of increasing the certainty of identification of a pesticide are described in Part I of this paper.

The first is by improvement of the resolution of a single packed column; the one described gives complete separation of at least 11 of the less volatile chlorinated pesticides in a run time of 30 minutes.

The second method involves similtaneous gas chromatography on five parallel columns holding stationary phases with differing gas - liquid chromatographic characteristics. In this system the eluate is fed to a single electron-capture ionisation detector. The multi-column "spectrochromatogram" produced has an appearance, and shows retention times, that are quite different and therefore highly characteristic for each individual pesticide. By this technique more certain identification is possible, *e.g.*, in such problem analyses as aldrin in interference-containing grain or soil, and dieldrin in admixture with *pp'*-DDE in animal tissues.

THREE years ago we indicated[1] that gas - liquid chromatography with detection by electron-capture ionisation was a technique with considerable potentialities in the field of pesticide-residue analysis, and followed this up by reporting procedures for the rapid identification and determination, on the nanogram scale, of the residues of several chlorinated pesticides in crops,[2] soils[3] and animal tissues[4] by this means. The exceptional sensitivity and selectivity of the electron-capture ionisation detector to such pesticides was also appreciated by Clark[5] and later by Watts and Klein,[6] by Moore[7] and by Taylor,[8] who successfully applied this technique to residue analyses on crops and animal products, foliage and soil, and animal and avian tissues, respectively.

Interest in residue analysis by electron-capture gas - liquid chromatography has since developed quickly in the laboratories of agricultural concerns, public health authorities and instrument manufacturers, particularly in the United States, and has resulted in a rapidly increasing volume of papers either just published,[9 to 15] or in the press.

There are several advantages of this technique over alternative methods of analysis of residues of chlorinated pesticides. Firstly, the gas - liquid chromatographic step makes possible simultaneous determination of two or more pesticides; secondly, the exceptional sensitivity of the detector allows analyses to be made, without concentration of the extract, on samples much smaller than could be used previously; and lastly, the selectivity of the detector to compounds of high electron affinity permits residues of the chlorinated pesticides to be determined in extracts of samples of biological origin with the minimum of prior clean-up.

In common with all other analytical methods, however, the technique of electron-capture gas - liquid chromatograph has its own particular limitations. One of these relates to the

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characteristics of the electron-capture ionisation detector, whose originator has recently given a timely warning[16] of the care needed in its application and use, if the results obtained are to be fully valid.

A more general limitation concerns the gas - liquid chromatographic step in which identification of the pesticide being analysed is normally based on the retention time produced on a single gas-chromatographic column. The shortcomings encountered here are that two pesticides may have the same retention time on one particular column or that one pesticide may have the same retention time as a naturally-occurring co-extracted product either having high electron affinity, or being present in massive amount. These failings are of particular relevance in screening analyses on samples of unknown history, when valid control material is not usually available. During the last year our efforts have therefore been directed towards resolving or, at least, alleviating this problem.

TABLE I

RESOLVING POWER OF GAS - LIQUID CHROMATOGRAPHIC COLUMNS GIVING SATISFACTORY CHROMATOGRAPHY OF A WIDE RANGE OF CHLORINATED PESTICIDES ON THE NANOGRAM SCALE

			Stationary phase				
Column length, feet	Column bore, inches	Celite mesh-size, B.S.S.	Silicone oil, % w/w	Epikote 1001, % w/w	Nitrogen flow-rate, ml per minute	Aldrin retention time, minutes	Aldrin - Telodrin resolution
2	0·065	100 to 120	2·5	0·25	35	3·25	None
2		120 to 150	2·5	0·25	80	4·25	None
4		60 to 72	10·0	1·00	100	7·30	0·75
6		100 to 120	2·5	0·25	40	6·90	1·00
10		60 to 72	2·5	0·25	45	7·50	1·15
4	0·095	60 to 72	5·0	0·50	120	4·40	0·95
4		60 to 72	2·5	0·25	30	6·50	0·95
6		60 to 72	2·5	0·25	115	4·45	1·10
2	0·125	100 to 120	2·5	0·25	60	4·00	0·55
2		100 to 120	5·0	0·50	115	4·70	1·10
4		60 to 72	2·5	0·25	100	4·55	1·10
4		100 to 120	2·5	0·25	100	6·00	1·30*
4		85 to 100	2·5	0·25	50	7·10	1·35
10		72 to 85	0·8	0·20	100	6·75	1·70
10		100 to 120	2·5	0·25	90	34·0	1·75
10		100 to 120	0·8	0·20	85	10·0	2·15
2	0·195	100 to 120	2·5	0·25	100	3·80	0·85†
2		100 to 120	2·0	0·50	100	3·40	0·90
4		100 to 120	0·8	0·20	100	4·50	0·90

* Illustrated in Fig. 1 B and Fig. 2.

† Illustrated in Fig. 1 A.

Resolution calculated from the expression

$$\text{Resolution} = 2 \times \frac{\text{difference between retention times}}{\text{sum of peak widths}}$$

There are three obvious ways by which certainty of identification of a pesticide can be increased in gas chromatography—

(*i*) improvement of column resolution,

(*ii*) application of stationary phases with differing characteristics or

(*iii*) complementary use of detectors having dissimilar responses.

Each of these approaches has been examined. The results of our work on the first two are reported in Part I of this paper, and on the last in Part II.

EXPERIMENTAL AND RESULTS

IMPROVEMENT OF RESOLUTION ON SINGLE COLUMNS—

Consideration of column design parameters—When considering ways in which the resolution of a packed gas-chromatographic column might be improved, attention was paid to the stationary phase, the supporting medium and also to the dimensions and material composition of the column itself.

For the stationary phase, no compound was found that would give a basic resolution superior to that of a non-polar silicone oil or elastomer.

As supporting medium, plain Celite, probably the most widely used material, was preferred. Although acid-washed Celite or other supports, *e.g.*, Diatoport, can be used with silicone oil alone, endrin may be at least partially decomposed at the nanogram level. With plain Celite, of course, the addition to the silicone of Epikote 1001, in 10 + 1 ratio, is necessary to avoid adsorption or decomposition effects. This small addition has no marked effect on the chromatographic characteristics of the silicone oil.

As a column material, copper has several advantages. It is cheap, robust and easily worked. It is certainly more reactive than borosilicate glass,[17] quartz[18] or polytetrafluoroethylene, but this disadvantage is obviated in the presence of the Epikote 1001 additive.

In developing conditions for improved resolution we imposed a limit of about thirty minutes for the gas - liquid chromatographic run-time, since protracted run-times reduce the attractiveness of the method and, moreover, increase the decomposition problem.

Run times can, of course, be reduced by decreasing the amount of stationary phase or increasing the column temperature or the gas flow-rate. The effect of marked reduction of the first would be to increase insecticide decomposition. This, coupled with increased vapour loss of the Epikote would also occur with the second. The gas flow-rate of 100 ml per minute at 163°C was already considered high by normal gas - liquid chromatograhic standards and was likely to be limited by increase in the drop of gas pressure when longer columns were used.

The two main variables remaining to be examined in the experiments on packed columns were, therefore, those of column length and bore.

Some consideration was also given to the application of capillary columns in this field of work. Such columns have found wide use in the petroleum and petrochemical industries because of their exceptional resolving power in the gas - liquid chromatography of relatively volatile materials, but little has been reported[19] on their value in pesticide residue analysis.

Evaluation of packed copper columns—Experiments were carried out to determine the resolution obtained, particularly for an aldrin - Telodrin* mixture, on packed copper columns varying in length from 2 to 10 feet and in bore from 0·065 to 0·195 inches. These columns were packed with various concentrations and ratios of silicone oil - Epikote 1001 mixtures as stationary phase, supported on plain Celite, ranging in mesh size between 60 to 72 and 120 to 150 (B.S.S.). The flow rate of nitrogen carrier gas was varied from 30 ml per minute to 120 ml per minute; the maximum inlet pressure used was 45 p.s.i.g. Column temperature was maintained at 163° C throughout.

The results obtained in these tests are summarised in Table I. Appraisal of these results and other relevant factors led to the conclusion that, within the run-time limit we imposed, the most suitable column for general use consisted of a 4-foot length of 0·125-inch bore copper tubing, packed with 2·5 per cent. w/w silicone oil plus 0·25 per cent. w/w Epikote 1001 supported on 100- to 120-mesh plain Celite maintained at 163° C and with a nitrogen flow-rate of 100 to 120 ml per minute.

A chromatogram of a mixture of aldrin, Telodrin, dieldrin and endrin run under the above conditions is shown in Fig. 1 B, and may be compared with a chromatogram of the same mixture, shown in Fig. 1 A, run on the standard 2 feet long × 0·195-inch bore type of column we were using[2] 2 years ago. It will be noted that with the new column, near-baseline resolution is achieved with both pairs of insecticides, though with an increase in run time of about 60 per cent.

The greater resolution of the new column permits satisfactory chromatography of a mixture of several more chlorinated pesticides (present only in nanogram amounts) than was previously possible. Fig. 2 shows a chromatogram in which thirteen of the less volatile pesticides are fully resolved in all but two instances.

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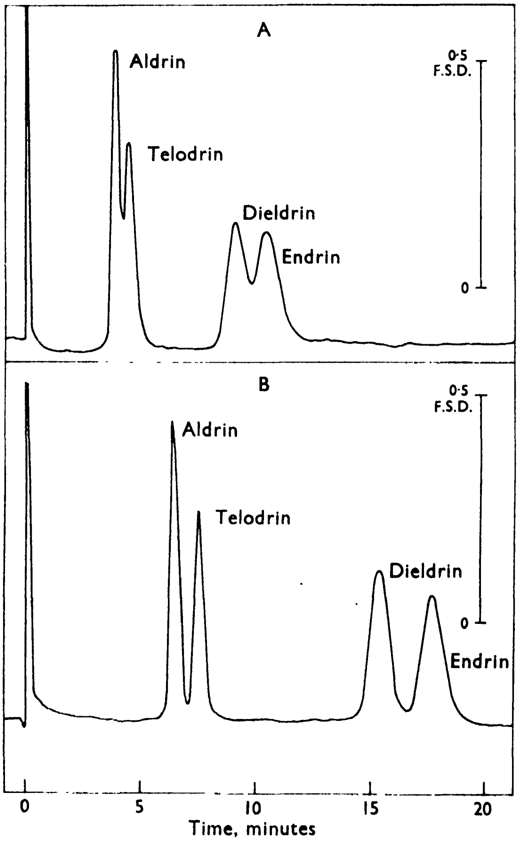
* Telodrin is the Shell Trade Mark name for 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

Evaluation of copper capillary columns—Most of the work on capillary columns was with copper as column material. The attractions here were, again, cheapness and ease of working. For example, no difficulty was encountered in obtaining gas-tight joints: this was not so when polytetrafluoroethylene was used as capillary tubing.

The columns tested had a bore of 0·018 inches and varied in length from 25 to 125 feet. They were coated with silicone oil, with or without added Epikote 1001, by means of the dynamic method involving a plug of stationary-phase solution.

Because of the very low concentration of pesticides present in the extract used, splitting of the 1 to 8 μl sample on injection was not possible. A polytetrafluoroethylene insert was, therefore, located at the inlet of the injection port to reduce its dead volume and thereby minimise diffusion.



A: Column dimensions, 2 feet long, 0·195-inch bore
B: Column dimensions, 4 feet long, 0·125-inch bore

Fig. 1. Electron-capture gas chromatogram, showing resolution of chlorinated-pesticide pairs on packed columns

A temperature of 163° C was used throughout, with nitrogen carrier-gas flow-rate ranging from 2 to 20 ml per minute. For detection, a planar-source electron-capture ionisation cell of low (0·1 ml) internal volume was used, and was fed with nitrogen scavenger gas to maintain an adequate flow rate (50 ml per minute) through the detector.

When a mixture of chlorinated pesticides, present in nanogram amounts, was analysed on the gas-liquid chromatographic column, decomposition was found to be appreciable except when Epikote was present. Further, in most experiments, resolution was much inferior to that obtained on normal packed columns.

The best capillary column prepared was 125 feet long and was coated with a 2 per cent. w/v solution of silicone oil and Epikote 1001 in a 10 + 1 ratio in ethyl acetate. This column, however, had a resolution only slightly better than that of the standard packed column used to produce Fig. 1 A and, moreover, effected no decrease in run time.

APPLICATION OF THE MULTI-COLUMN "SPECTROCHROMATOGRAM"—

General considerations—In earlier papers[2,4] we drew attention to two ways in which uncertainty of identification of a pesticide, caused by the presence of interfering material of coincident retention time, might be overcome. These were to effect resolution of the two components by use of a second (polar) column, whose gas - liquid chromatographic characteristics differed from the standard (non-polar) column; or, alternatively, to remove the interfering component previously by liquid - liquid chromatography.

The latter technique is not always successful[14] and is of limited value in screening analyses on samples of unknown history, since loss of other sought-for pesticides or their metabolites may occur during the process.

The first-mentioned technique was pursued in the Tunstall Laboratories of "Shell" Research Ltd. by Robinson and Richardson,[20] who determined the relative retention of eight chlorinated pesticides on single columns of Apiezon L and Nonidet P40 as well as the silicone elastomer E301 and Epikote 1001 used by ourselves.

We therefore examined the usefulness of simultaneous gas liquid chromatography on multiple parallel columns, each containing stationary phases of differing characteristics, as a method for increasing the certainty of identification of pesticides, particuarly when these suffer interference from other pesticides or from naturally-occurring co-extracted material having coincident or near-coincident retention times on a standard (non-polar) column. The chromatograms so produced we have tentatively called "spectrochromatograms," because they are indicative of the spectrum of selectivity of the various stationary phases towards individual chlorinated pesticides.

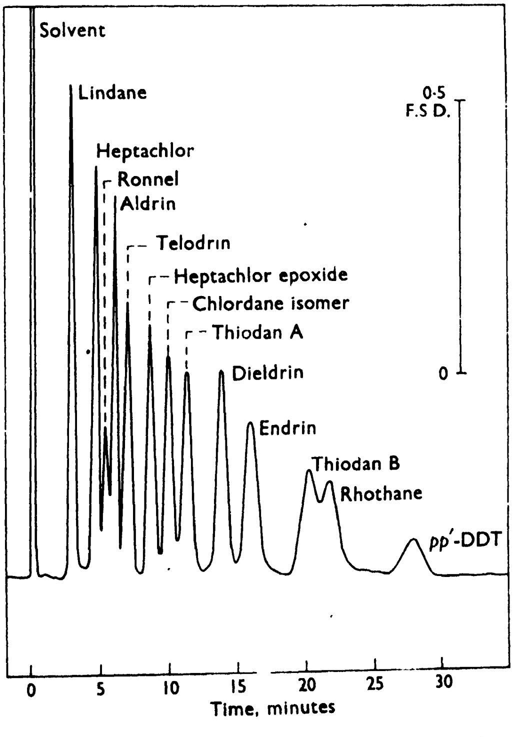


Fig. 2. Electron-capture gas chromatogram, showing resolution of thirteen chlorinated pesticides on a packed column: 0·4 nanograms of each pesticide

Requirements for, and evaluation of, stationary phases—It was decided to develop a multi-column system having five stationary phases; this was the maximum number that it was felt could reasonably be handled operationally. These five phases would ideally comprise two or three of proven general applicability in the chlorinated pesticide field, while the others would be present mainly because of their ability to resolve obvious problem pairs, *e.g.*, aldrin and grain interference or dieldrin and *pp'*-DDE mixtures.

The aim in searching for suitable stationary phases was that they should cover the widest possible range of polarity and structure, should be stable at the operating temperature of 163° C and, in particular, exhibit insufficient bleed to affect the highly sensitive electron-capture ionisation detector.

In addition to the silicone oil, Epikote 1001 and Apiezon L already referred to, twenty-one materials were evaluated as potential stationary phases on plain Celite for the gas - liquid chromatography of a test mixture of seven chlorinated pesticides. These pesticides were: indane, heptachlor, aldrin, Telodrin, dieldrin, endrin and *pp'*-DDT.

The stationary phases chosen are grouped below under three headings:

1

Behenic acid
Calcium gluconate
D(+)Glucose
Mannitol hexa-acetate
Polyglycerol
Sodium carboxymethylcellulose

2

Adipic acid
Araldite
Mannitol
Nitrile silicone XF-1150
Nonidet P40
P.E.G. 4000
P.E.G. 6000
P.E.G. 15-20,000
Zinc stearate

3

Diethylene glycol succinate
Dulcitol
Nitrile silicone GE-XE-60
Nitrile silicone XF-1112
Salicin
D(−)Sorbitol

The first group (1) was quite unsatisfactory (giving little or no chromatography); the second group (2) was partially successful (chromatography of 3 or 4 pesticides); while the last group (3) was the most successful (chromatography of 5, 6 or 7 pesticides).

It was clear from earlier work[2,20] that addition of Epikote 1001 to either the silicone or Apeizon stationary phases on plain Celite was essential if adsorption or decomposition of some of the chlorinated pesticides was to be avoided. The effect of this additive on salicin, nitrile silicone GE-XE-60 and diethylene glycol succinate was therefore examined. Only with the latter compound was there significant improvement in chromatography, although even here partial loss of heptachlor and total loss of *pp'*-DDT still occurred.

This work led to a consideration of other ways in which such adsorption - decomposition effects could be overcome. Pre-treatment of the stationary phase and supporting medium with tris-(2-biphenylyl) phosphate (Dow K-1110) was reported by Gunther, Blinn and Kohn,[21] but the success of this method was not confirmed by Beckman and Bevenue.[18] As an alternative to Epikote 1001, we found that Araldite, a proprietary epoxy resin adhesive, worked as well for the above purpose. The use of such epoxides resulted in experiments on the pre-treatment of plain Celite with epichlorhydrin under reflux. It was found that Celite so treated permitted the test mixture of the seven pesticides to be analysed by gas-liquid chromatography on the nanogram scale without decomposition, when silicone alone was used as stationary phase. This work has yet to be further investigated.

Design, composition and operation of the multi-column assembly—A multi-column assembly head was constructed in brass. The injection port led via a short capillary manifold to the inlets of five parallel U-shaped copper columns each 4 feet long with a 0·125-inch bore. The outlets of these five columns were then led via a second short capillary manifold, also located in the assembly head, to the inlet of a single planar-source electron-capture ionisation detector, whose internal volume was about 0·1 ml. All column connections were made with Simplifix fittings.

In preliminary tests severe decomposition of some pesticides was traced to contact with the metal of the assembly head. This decomposition was suppressed by washing the head out with a 2 per cent. w/v solution of silicone oil and Epikote 1001 mixture, in 4 + 1 ratio in ethyl acetate, followed by evaporation of the solvent before attaching the columns.

The final choice of five stationary phases, based on the requirements and evaluations given earlier, is given below, together with the concentrations used. These were carefully chosen after appreciable experimentation to give peak resolution of as many chlorinated pesticides, metabolites and artifacts as possible.

(*a*) 2·5 per cent. w/w silicone oil (May and Baker Ltd.) + 0·25 per cent. w/w Epikote 1001 (Shell Chemical Co. Ltd.).

(*b*) 1·0 per cent. w/w Apiezon L (A.E.I. Ltd.) + 0·2 per cent. w/w Epikote 1001.

(*c*) 1·0 per cent. w/w Epikote 1001.

(*d*) 3·3 per cent. w/w nitrile silicone fluid XF-1112 (General Electric Co., U.S.A.).

(*e*) 2·7 per cent. w/w nitrile silicone fluid GE-XE-60 (Applied Science Labs. Inc.; U.K. Agents, W. G. Pye & Co. Ltd., Cambridge).

The procedure for residue analysis with the multi-column assembly was essentially the same as that described previously for single column operation, except that injection volumes were increased fivefold to 25 µl to compensate for splitting of the charge into the parallel columns. The crop, soil or tissue sample was macerated with the minimum volume of

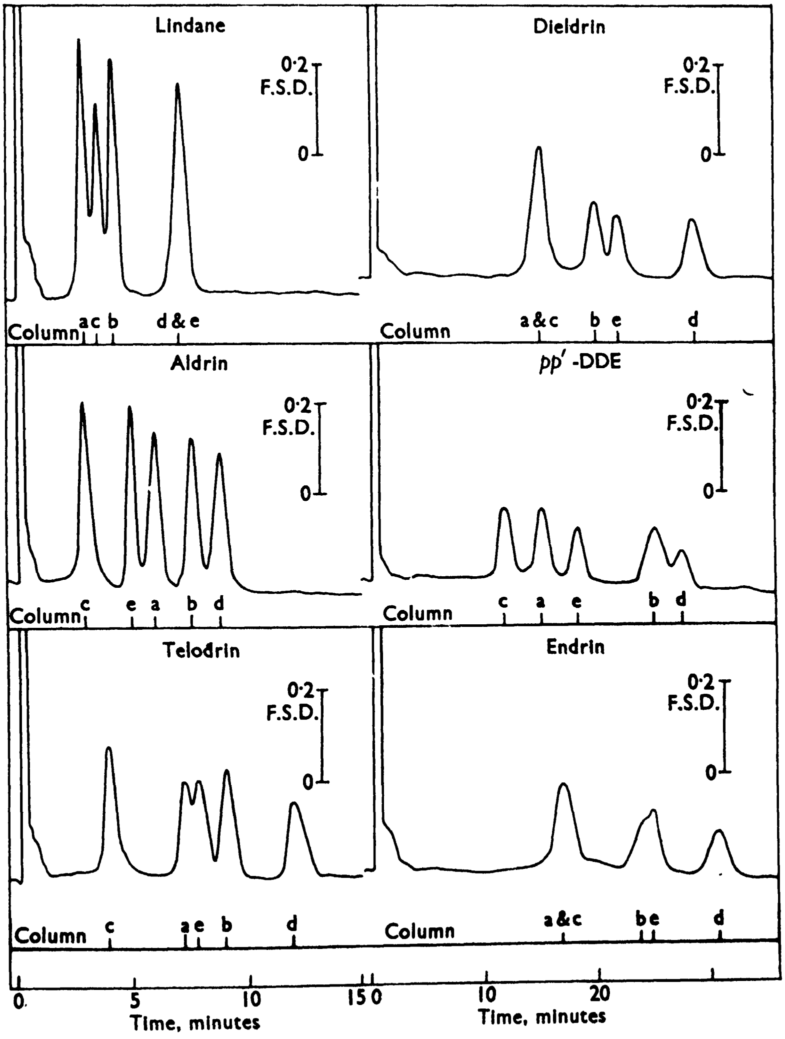


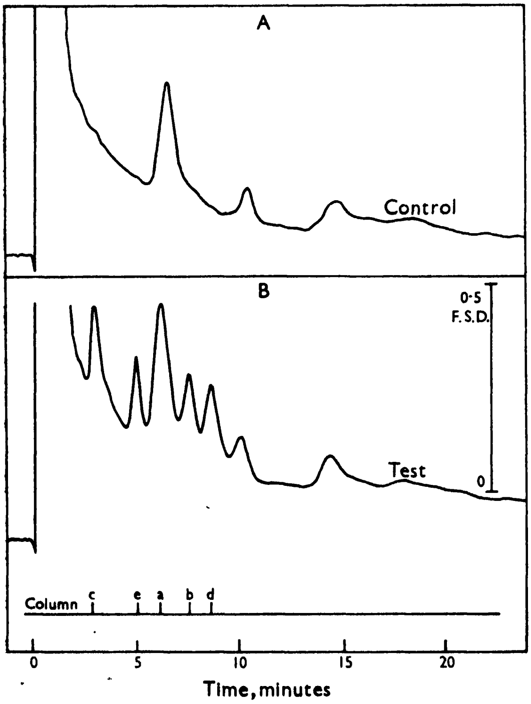
Fig. 3. Multi-column "spectrochromatograms" of some chlorinated pesticides: injection, 25 µl = 2·5 nanograms of each pesticide

redistilled analytical-reagent grade acetone to give a fluid macerate that was filtered through sintered glass. The filtrate was made up to two volumes per unit weight of sample. A portion of this acetone extract was then shaken with twice its own volume of redistilled petroleum spirit (boiling-range 62° to 68° C), in the presence of excess aqueous sodium sulphate solution. This petroleum-spirit extract was used for the injection.

Column temperature was maintained at 163° C, with the nitrogen carrier-gas flow-rate through each column at the normal rate of 100 ml per minute. The flow-rate was set at this level to keep retention times as short as practicable, although this resulted in a very high combined flow-rate of 500 ml per minute to the detector, which the latter accepted without malfunction.

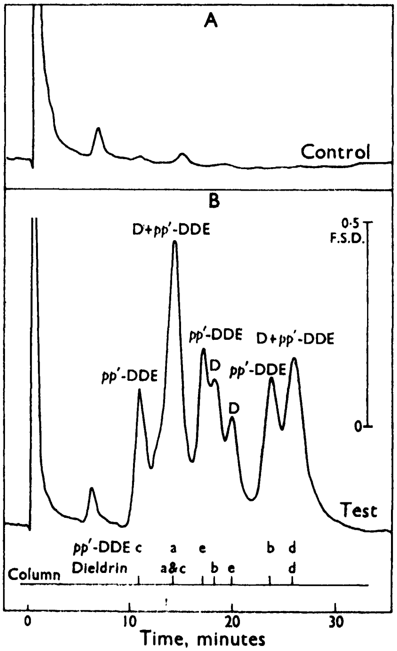
Evaluation of "spectrochromatograms" and multi-column performance—By using the above-mentioned system, multi-column "spectrochromatograms" were produced for a range of the less volatile chlorinated pesticides. Those for lindane, aldrin, Telodrin, dieldrin, *pp*′-DDE and endrin are shown together in Fig. 3. In general, 3 to 5 distinct peaks are obtained and show retention times which, considered together, provide good evidence for the identification of the compound present. For example, on our standard silicone and Epikote column of two years ago (see Fig. 1 A), aldrin was only partially resolved from Telodrin: in Fig. 3, however, it will be seen that the "spectrochromatogram" of aldrin is quite different both in appearance and indicated retention time from that of Telodrin. Similarly, on the standard column dieldrin was only partially resolved from endrin and not at all from *pp*′-DDE: Fig. 3 shows their "spectrochromatograms" to be appreciably different.

Most grain crops (wheat, oats, barley, maize and rice) have been found to contain materials that have the same retention time as aldrin on the standard column. The results of applying the multi-column system to this problem are shown in Fig. 4 in which, despite the presence



A: Control oats
B: Oats containing 0·25 p.p.m. of aldrin

Fig. 4. Multi-column "spectrochromatogram" of control and aldrin-treated oats: injection, 25 μl ≡ 6 mg of crop



A: Control beef
B: Beef containing 1·0 p.p.m. of *pp*′-DDE and 0·5 p.p.m. of dieldrin

Fig. 5. Multi-column "spectrochromatogram" of control beef and beef containing dieldrin and *pp*′-DDE: injection, 25 μl ≡ 6 mg of tissue

of the naturally-occurring interference, peaks whose appearance and retention times are characteristic of the aldrin "spectrochromatogram" are clearly evident in the aldrin-treated sample. Similar results were obtained on interference-containing soil samples.

Another problem is the one in which two insecticides that are unresolved on the standard column may well be present together in the test sample. Such an example is that of dieldrin and *pp'*-DDE in animal tissues. The "spectrochromatogram" produced for this mixture is shown in Fig. 5, in which it can be seen that there is sufficient characterisation to identify these two pesticides.

The reproducibility of "spectrochromatogram" retention times was found to be as good as on single columns. Reproducibility of splitting of the injection charge between the five parallel columns was, however, less satisfactory and was thought to be limited by the design of the particular assembly head used.

The life of the multi-column assembly was found to be restricted to about two weeks owing to ageing or vapour loss of some of the stationary phases, in particular the nitrile silicone XF-1112 and Epikote 1001. These effects resulted in an increase in pesticide decomposition with time coupled with changes in the retention times shown by the "spectrochromatogram" peaks. In consequence it was found advisable to determine the reference "spectrochromatograms" at the same period of time as those of the test samples were being run.

An indication of the value of the multi-column "spectrochromatogram" technique in screening work was obtained inadvertently when a wide variety of locally purchased "control" crops was being examined for use in work on the complementary detection system to be reported in Part II of this paper. Clearly-defined "spectrochromatograms" showed traces of identifiable chlorinated pesticide in one or two of these samples.

Discussion and conclusions

The development work on single packed columns described earlier in this paper produced a useful though not marked gain in resolution in a column having a general use for screening purposes. In consequence the 4 feet × 0·125-inch bore column is to be preferred to the 2 feet × 0·195-inch bore column used previously. It does not, however, greatly improve the certainty of identification of any particular pesticide. Against this may be set the fact that it had to be designed to give rapid chromatography of nanogram amounts of a very wide range of chlorinated pesticides (see Fig. 2). In more specialised circumstances, for example, when longer run times are no detriment, a valuable increase in resolution can be achieved by using 0·125-inch bore columns of much greater length.

The brief study of capillary columns proved disappointing, considering the known value of these columns in other fields of work. The main problem here lies in the extremely low dilution of the extract solution being analysed, rendering the stream splitting normally used in capillary work impracticable. There remains, nevertheless, much scope for further development in the application of capillary columns to pesticide-residue analysis, not only on solving the above-mentioned injection problem but in evaluating columns of other bores, lengths and materials and different stationary-phase systems.

As a means for improving the certainty of identification of residues of the chlorinated pesticides, the multi-column "spectrochromatogram" technique showed more promise than the others referred to, and the method could well be applied as a supplementary confirmatory technique for those samples that had given an indication on a short primary screening column of the possible presence of pesticide. The multi-column assembly is, of course, more troublesome to prepare, has a shorter working life than the single column and, at present, is only roughly quantitative. Against this, however, the method is capable of further improvement. Re-design of the assembly head, possibly in stainless steel, aluminium or glass may reduce the tendency for pesticide decomposition inside it, thus avoiding the need for the silicone and Epikote pre-treatment. At the same time, an improvement of the reproducibility of splitting of the charge into the parallel columns might be achieved. This would further increase certainty of pesticide identification by giving more reproducible "spectrochromatograms," and would also improve the extent to which the technique may be used quantitatively. A further contribution of value would be the introduction of alternative stationary phases; the two main requirements here are thermally stable and highly polar compounds.

We thank Mr. D. M. Barnett for much careful experimentation, Mr. G. F. Poulton for the design and construction of the multi-column assembly, and Dr. R. A. E. Galley, Director, and Mr. J. G. Reynolds, Associate Director of the Woodstock Agricultural Research Centre, for their interest and encouragement during the course of this work.

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Improvement of Identification in the Gas - Liquid Chromatographic Analysis of Agricultural Samples for Residues of some Chlorinated Pesticides*

Part II. A Halogen-sensitive Detector in Complementary or Alternative Use to an Electron-capture Ionisation Detector

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("Shell" Research Ltd., Woodstock Agricultural Research Centre, Sittingbourne, Kent)

Gas - liquid chromatography with detection by electron-capture ionisation has found considerable use in the analysis of crops, soils and animal tissues for residues of the chlorinated pesticides. The chief limitation of the method, however, lies in incomplete certainty of identification of a pesticide based on retention time alone on a single column of the resolving power at present available.

Two methods of increasing certainty of identification, the use of single columns of higher resolving power and the application of the multi-column "spectrochromatogram," were described in Part I of this paper.

In Part II, a third method based on simultaneous detection by two highly sensitive and selective detectors with dissimilar response characteristics to individual chlorinated pesticides is described. One of these sensors is the electron-capture ionisation detector, and the other is a halogen-sensitive cell of the type used in refrigeration leak detection. The last-mentioned detector has great potential value in residue analysis for the chlorinated pesticides. It is sensitive to the nanogram range, is more selective than the electron-capture ionisation type and has greater linearity of response. Moreover, it is relatively cheap and can be operated with simple circuitry.

GAS - LIQUID chromatography with detection by electron-capture ionisation has rapidly proved itself[1,2,3,4] to be a technique of particular value for detecting and determining residues of chlorinated pesticides in crops, soils and animal tissues. Its main advantages over alternative methods are greater sensitivity, greater selectivity and its ability to analyse for more than one component at the same time. These advantages result in a method in which extracts of small samples can be analysed rapidly for nanogram amounts of several pesticides without a concentration step, and with the minimum of prior clean-up.

Although care is necessary in the proper operation[5] of the detector itself, the main limitation of the method lies in the degree of certainty of identifying the individual pesticides, when this identification is based only on retention times obtained on a single column of the resolving power at present available. This factor is most relevant in screening analyses on samples of unknown history, when valid control material is not usually available.

In Part I of this paper,[6] two methods intended to overcome or reduce this problem were described: the use of single columns of higher resolving power than that of earlier versions, and the production of multi-column "spectrochromatograms" having an appearance and showing retention times highly characteristic of the individual pesticides being analysed.

A third approach, using highly sensitive detectors whose responses to the halogenated pesticides differ, is described in Part II. A detector is needed that has the unusual properties of exceptional sensitivity and high selectivity to the halogenated pesticide, but significantly different response characteristics from the electron-capture ionisation detector with which it could be used in tandem. Such a device is the halogen-sensitive element used in the Type HA leak-detector made by Associated Electrical Industries Ltd.

The idea of applying a halogen-sensitive device of this type to the analysis of pesticide residues is not new, for in 1952. C. A. Reilly, working in the Laboratories of the Shell Development Company at Emeryville, California, reported on a determined effort to use the General Electric Type H leak-detector of similar design to the Type HA for this purpose. This work, however, was not altogether successful, partly because the use of the detector was not preceded by a gas - liquid chromatographic step, and partly because of the electrical or electronic instability of the systems examined.

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* A summary of this paper was presented at the Vth International Pesticides Congress, London, July 17th to 23rd, 1963.

Much more recently the successful use of this type of halogen-sensitive element as a detector in the gas - liquid chromatography of volatile chlorinated hydrocarbons was briefly reported by Cremer, Kraus and Bechtold[7] in Germany. In this detailed study, high selectivity to chlorinated compounds was recorded, and such a sensitivity was developed that 0·3 nanograms of chlorine could be detected under favourable conditions.

This lead was followed in the Tunstall Laboratory of "Shell" Research Ltd. by A. Richardson, who showed that the A.E.I. halogen-sensitive element already referred to would function as a gas - liquid chromatographic detector, and could provide a relatively high sensitivity with the aid of only simple circuitry.

We therefore undertook a detailed evaluation of two versions of this halogen-sensitive device with the object of using it for determining residues of the chlorinated pesticides on the nanogram scale, and of operating it in tandem with an electron-capture ionisation detector as a method for increasing the certainty of identifying these compounds.

EXPERIMENTAL AND RESULTS

DESCRIPTION AND OPERATION OF THE HALOGEN-SENSITIVE ELEMENT—

The A.E.I. Ozotron Type H halogen-sensitive element,[8] shown diagrammatically in Fig. 1, consists of a pair of concentric platinum cylinders mounted within a protective borosilicate glass envelope through which, in normal operation, the air to be tested for traces of halogenated compounds is drawn at a rate of about 150 ml per minute. The inner platinum cylinder, or anode, which has been sensitised by an alkali treatment, is indirectly heated by an internal platinum filament taking a current of 7·4 amps at 6 volts a.c., and operates at a temperature of about 800° C. An anode-to-cathode potential of 250 volts d.c. is normally used, resulting in the production of a positive-ion standing current that can be suitably amplified and presented. When air, containing halogen vapour, is drawn through the cell there is an apparent increase in the positive-ion current, whose magnitude is indicative of the concentration of halogen vapour present.

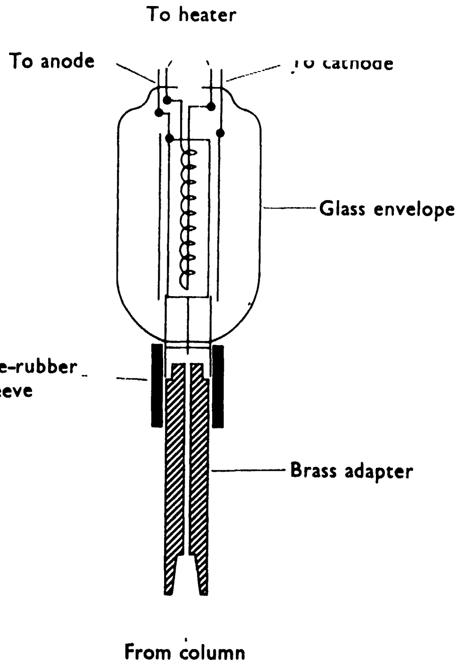


Fig. 1. Halogen-sensitive detector (A.E.I. type H)

A newer version of the above detector has recently become available. This model, the Ozotron Type J, is basically the same as the Type H except that the element is mounted more rigidly in a two-piece ceramic envelope from which the platinum heater filament and electrodes are easily removed.

Design of circuits and performance of halogen-sensitive detectors—

An Ozotron Type H halogen-sensitive element was connected up as detector to a 4 feet long × 0·125-inch bore silicone - Epikote column of the type described in Part I of this paper. The detector circuitry used is shown diagrammatically in Fig. 2(*a*). Power for the heater filament was supplied from a 240 to 6V step-down transformer connected in series with a 1-ohm sliding resistance, and the potential required for the anode was taken from 0 to 120 V high tension batteries. The signal from the cathode was led, via a 1-megohm load in series with a 1500-ohm input resistor (backed off by a 0 to 9 V grid-bias battery controlled by a wire-wound variable resistor), before being led directly into a 1-mV full-scale deflection chart recorder having a maximum input impedance of 1000 ohms and a pen-response time of 2 seconds.

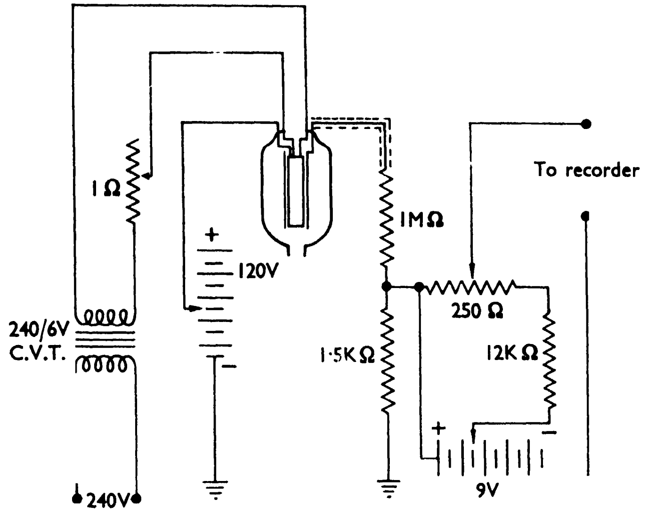


Fig. 2 (*a*). Simple circuit for halogen-sensitive detector

An evaluation of detector performance was made under these conditions by injecting 1 to 10 μl volumes of dilute solutions of chlorinated pesticides in light petroleum (boiling-range 62° to 68° C) into the gas - liquid chromatographic apparatus at a column temperature of 163° C, while varying the operational parameters. Nitrogen was used as carrier gas at various flow-rates in the range 100 to 200 ml per minute, with and without the admission of air at the inlet of the detector.

It was found preferable to use separate leads to the anode and inner heater terminal instead of a common one as indicated by the manufacturer, as the latter procedure resulted in a higher electrical noise level.

Sensitivity, both to chlorinated pesticides and to light-petroleum solvent, increased with increase in heater voltage, but in favour of the solvent. In consequence it was impracticable to work at the highest heater voltage (6 volts), as the large solvent response obtained masked the early part of the chromatogram. Below about 4 volts, however, sensitivity rapidly decreased.

Similarly, increase in the potential applied to the anode of the cell resulted in an increase in both sensitivity and electrical noise level. Disproportionate increase of the latter made it desirable to operate at comparatively low potentials, although below about 36 volts d.c. a marked increase in peak tailing seriously affected the chromatograms obtained.

The time-constant of the cell was observed to be rather longer than that for the electron-capture ionisation type of detector, and in consequence, higher nitrogen flow-rates (greater than 100 ml per minute) resulted in improved chromatogram peak shape, but at the expense of some sensitivity. Admission of air to the inlet of the detector to effect oxidative combustion of the pesticides rather than pyrolysis, produced a marked increase in the standing current of the cell and a reduction of sensitivity that became significant at air flow-rates in excess of about 5 ml per minute.

The level of sensitivity obtainable by using the above-mentioned simple circuitry and with a power input to the heater filament of 4·6 volts a.c. at 6·7 amps, an anode potential of +36 volts d.c. and a nitrogen carrier-gas flow-rate of 100 ml per minute at 163°C, corresponded to a limit of detection of about 2 nanograms for aldrin (approximately twice the noise level). Response of the detector *versus* pesticide load was observed to be linear up to recorder full-scale deflection, which for aldrin occurred with 100 nanograms.

This sensitivity, while encouraging, was not quite sufficient for our purpose, and a more sensitive version of the circuit, shown diagrammatically in Fig. 2(*b*), was therefore developed.

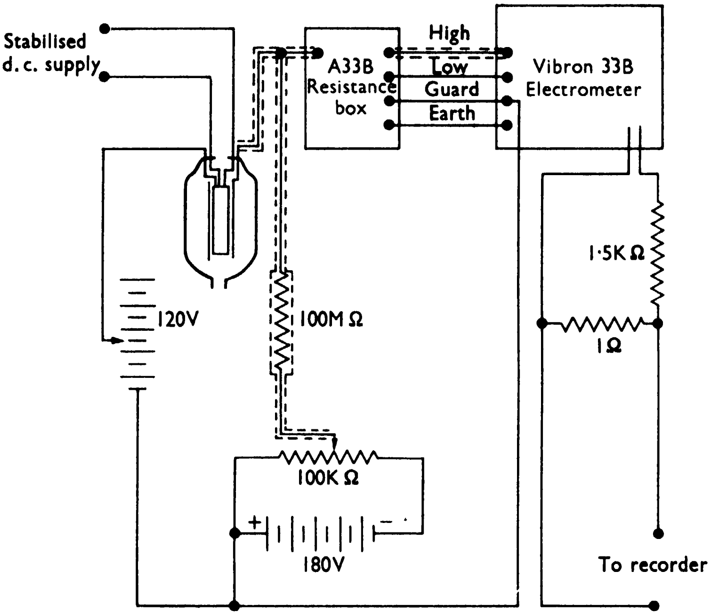


Fig. 2 (*b*). More sensitive circuit for halogen-sensitive detector

With this arrangement it was found essential to provide the heater filament with a more stable power supply than was given by the step-down transformer. Replacing the latter by a heavy duty 6 volt accumulator proved satisfactory, except that the high current consumption resulted in a gradual drift of the recorder baseline as the accumulator was discharged. The latter was therefore replaced by a comparatively inexpensive transistorised Zener-diode stabilised d.c. power supply, specially designed for the purpose.

In this more sensitive circuit the signal from the cathode was led through a 1-megohm input resistor in an Electronic Industries Ltd. model A33B current- and resistance-measuring bridge, backed off similarly to the one described above, and then passed into an E.I.L. Vibron model 33B vibrating-reed electrometer, before being fed via appropriate series - parallel matching resistors to the 1-mV recorder.

Under the same operating conditions as described above, the limit of detection for aldrin with this circuitry was about 0·1 nanograms at the 100-mV attenuation setting. Again, good linearity of response with increase in pesticide load was observed up to recorder full-scale deflection at the 1000-mV (maximum) attenuation setting, which for aldrin occurred with 30 nanograms.

A similar evaluation of the ceramic-protected Ozotron Type J halogen-sensitive element was also undertaken. The performance characteristics observed were similar in some respects to those outlined above.

In this work it was found essential to ensure that all signal-carrying leads were fully screened by using co-axial cable, and that electrical connections, particularly the leads to the heater elements, were soundly made, *e.g.*, brazed or silver-soldered.

The two least satisfactory features of the Type H and J detectors when used in the manner described, were their general electrical instability and the need for conditioning them appreciably before high sensitivity could be achieved. The tendency towards electrical instability was considered to be caused partly by some lack of rigidity in the location of the concentric platinum electrodes (in the Type H) and partly by shorting of the electrodes, perhaps by carbonaceous deposits resulting from pyrolysis of the samples. Such deposits could easily be removed by cleaning the electrodes in acetone; this was facilitated for Type J by the ease with which the cell could be taken apart.

The conditioning of the cells and their maintenance in a state of high sensitivity was the biggest problem encountered, and has not yet been completely solved. A variety of conditioning processes were tried with five such cells, including pre-treatment with air, nitrogen or massive loads of chlorinated material, and washing the anode and gentle abrasive cleaning of the cathode in acetone. Of these, pretreatment with air shows promise of being the most effective. The results quoted above were obtained on the most sensitive of the detectors examined and this one (a Type H) was used to produce the halogen-detector chromatograms reproduced in this paper.

EVALUATION OF HALOGEN-SENSITIVE AND ELECTRON-CAPTURE IONISATION DETECTORS IN COMPLEMENTARY OPERATION—

An Ozotron Type H halogen-sensitive element was connected by means of a short brass capillary tube and silicone rubber sleeve on to the outlet port of an electron-capture ionisation detector. This, in turn, was connected to a 4 feet × 0·125-inch bore copper column that was packed with 2·5 per cent. w/w of silicone oil *plus* 0·25 per cent. w/w Epikote 1001, supported on 100- to 120-mesh plain Celite. The column was operated at a temperature of 163° C, and a nitrogen flow-rate of 100 ml per minute was maintained. For the Ozotron, the more

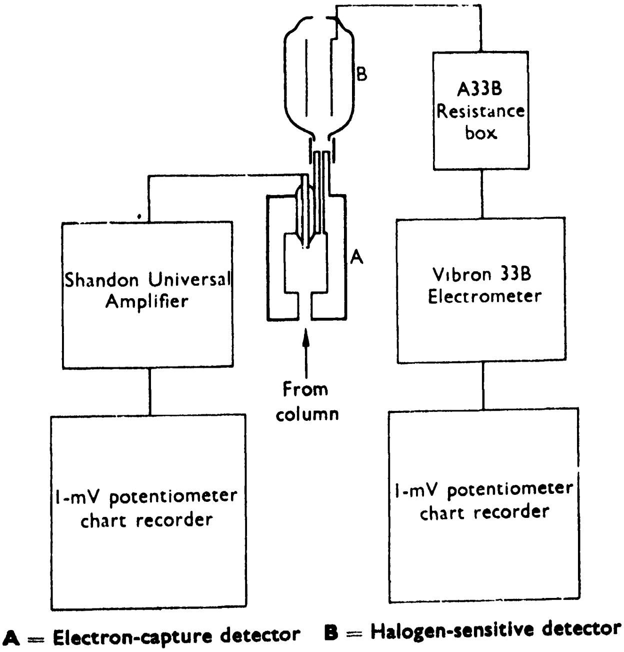

B
A33B Resistance box
Shandon Universal Amplifier
A
Vibron 33B Electrometer
From column
1-mV potentiometer chart recorder
1-mV potentiometer chart recorder
A = Electron-capture detector B = Halogen-sensitive detector

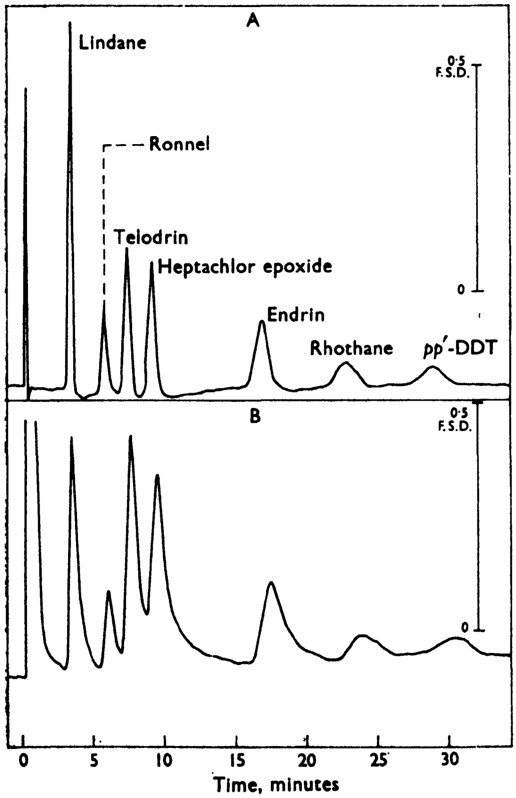
Fig. 3. Simultaneous complementary detection system

sensitive circuit was used, and the signals from both detectors after attenuation or amplification as appropriate were fed, as shown in Fig. 3, into separate 1-mV recorders running at the same chart speed. By this arrangement simultaneous chromatograms from these two different detection systems could be produced for solutions containing nanogram amounts of the chlorinated pesticides.

An example of this is illustrated in Fig. 4, in which the simultaneous halogen-sensitive and electron-capture chromatograms were produced on the injection of 2·3 μl of a petroleum spirit solution, containing 2·3 nanograms each of lindane, ronnel, Telodrin, heptachlor epoxide, endrin, Rhothane and *pp*'-DDT. It will be noted that the relative response of the two detectors is different, particularly for lindane, Telodrin and endrin.

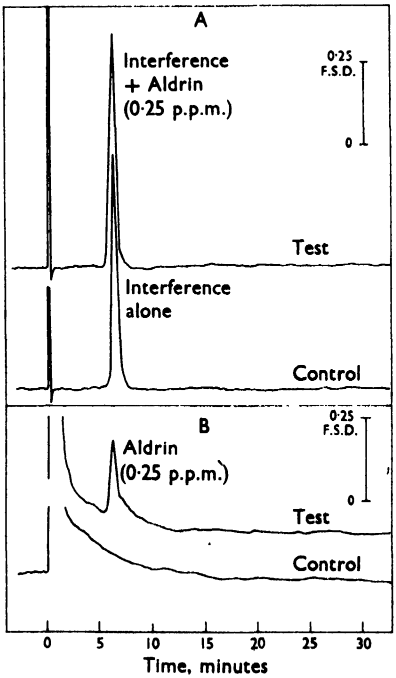
This difference in relative response can be more marked when sensitivity to chlorinated and non-chlorinated material is considered. In the top half of Fig. 5, the electron-capture chromatogram of "control" wheat before and after the addition of 0·25 p.p.m. of aldrin can be seen. As has been previously pointed out,[1] this control sample, typical of the grain crops, exhibits massive electron-capture interference from a naturally occurring co-extractive, whose retention time is the same as that of aldrin. In the simultaneous halogen-sensitive chromatograms, no such interference is evident. The complementary use of the two detectors could therefore be of much value in increasing the certainty of identification of any pesticide indicated as present in the sample.

The value of the difference in relative response of the two detectors to individual pesticides is shown in Fig. 6, in which, under standard operating conditions, reproducible relative responses (as measured by peak area ratios) provide good confirmatory evidence for the



A: Electron-capture detector
B: Halogen-sensitive detector

Fig. 4. Simultaneous complementary gas chromatograms of seven chlorinated pesticides: 2·3 nanograms of each pesticide.



A: Electron-capture detector
B: Halogen-sensitive detector

Fig. 5. Simultaneous complementary gas chromatograms of control and aldrin-treated wheat: injection, 5 μl ≈ 6 mg of crop.

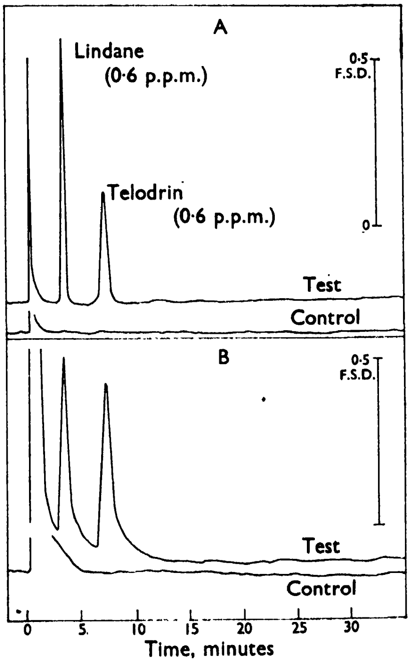
identification of the pesticides lindane and Telodrin, added to spring cabbage to the extent of 0·6 p.p.m.

In the last-mentioned chromatogram, the area of the halogen-sensitive detector peak for Telodrin (chlorine content, 68·8 per cent.) is greater than that for lindane (chlorine content, 73·1 per cent.), indicating that, for this detector, the response obtained is not simply a function of the chlorine content of the molecule.

Another example of simultaneous complementary halogen-sensitive - electron-capture gas - liquid chromatography is illustrated in Fig. 7, which shows the dual chromatograms produced by the addition of 1 p.p.m. of either dieldrin or *pp*′-DDE to "control" lamb. The retention times of these two pesticides on a single silicone - Epikote column are nearly coincident, with the consequent possibility of confusion between them. With the complementary detector system, however, the calculated peak area ratios, 1·3 and 1·1 for dieldrin and *pp*′-DDE respectively, could provide sufficient evidence to permit reasonable distinction between the two pesticides.

DISCUSSION AND CONCLUSIONS

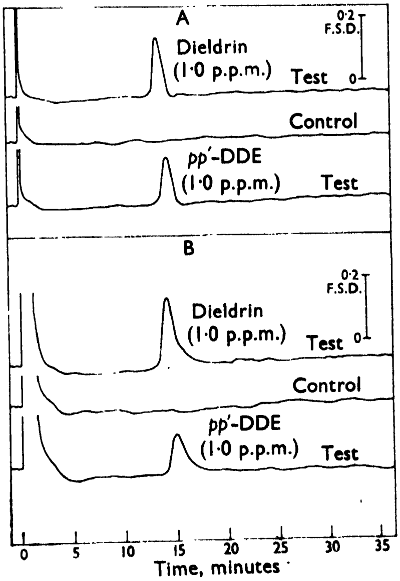
One of the chief merits of the halogen-sensitive detectors described lies in their exceptional selectivity towards the halogenated pesticides compared with natural materials co-extracted from crops, soils or tissues. There is evidence that this selectivity is significantly superior to that shown by the electron-capture ionisation type of detector. This is particularly marked in the example shown in Fig. 5. Certainly, in the screening of a wide range of crops with the halogen-sensitive detector, no sample containing non-pesticidal material gave a response greater than that for electron-capture ionisation detection, and there were indications that in general the superiority in selectivity was about tenfold.



A: Electron-capture detector (EC)

B: Halogen-sensitive detector (HS)

Fig. 6. Simultaneous complementary gas chromatograms of control and lindane - Telodrin treated spring cabbage: injection, 5 μl ≡ 6 mg of crop. Relative peak areas, HS/EC: lindane 1·1; Telodrin 2·5



A: Electron-capture detector (EC)

B: Halogen-sensitive detector (HS).

Fig. 7. Simultaneous complementary gas chromatograms of control lamb and lamb containing dieldrin or *pp*′-DDE: injection, 5 μl ≡ 2·5 mg of tissue. Relative peak areas, HS/EC: dieldrin 1·3; *pp*′-DDE 1·1

Another feature in favour of the halogen-sensitive element is that there is linearity of response with increase in load up to at least 100 nanograms. This could be of advantage in quantitative work, by reducing the need for repeated dilution and injection in the analysis of extracts of unknown pesticide concentration.

The cost of these detectors (£15 to £18) is not high, and in tests where the highest sensitivity (0·1 nanogram) is not required, simple and inexpensive circuitry that does not involve the use of either an electrometer or amplifier is adequate. This arrangement could be of immediate value in analyses in the near-residue range, *e.g.*, for chlorinated pesticides in field-strength dusts, fertilisers, wool, wood, plastics and hardboard, and after a tenfold concentration of the sample extract (by partition or evaporation, or both), could be applied to the low residue range. Indeed, it is probable that this concentration step can be eliminated simply by using a larger input resistor, and feeding the signal directly into a high-impedance 1-mV recorder.

The main disadvantages of these halogen-sensitive detectors, as has been already indicated, lie in their indifferent electrical stability under the conditions used, and the difficulty of conditioning the cells rapidly and maintaining them at high sensitivity. It must be remembered, however, that the detectors have been used in a manner quite unlike the one for which they were designed. It is possible, therefore, that design and development work on halogen-sensitive elements for use in gas - liquid chromatographic residue analysis could overcome both of these problems and, moreover, effect sufficient increase in sensitivity to make them of even greater value than the electron-capture type.

Most of the work reported here was performed with the Ozotron Type H detector, but the Type J shows considerable promise. The unglazed ceramic sheath of the latter, however, may not be as impervious as the glass of the former. Against this, the Type J is easier to clean and, perhaps, to condition since it can be taken apart. Further, it possesses a higher thermal capacity, which makes it somewhat less sensitive to slight fluctuations in the power supply to the heater filament.

In conclusion, it is considered that the halogen-sensitive detector when used in combination with the electron-capture ionisation detector in the manner described, provides a technique with a potential value at least comparable with that of the multi-column "spectrochromatogram" (described in Part I of this paper), for improving the certainty of identifying chlorinated pesticides in analysis of residues. Apart from its complementary operation with the electron-capture ionisation type, it has great potential value as an alternative detector in gas - liquid chromatographic analysis of chlorinated pesticides, both on the residue and near-residue scales.

We thank Mr. A. Richardson of the Tunstall Laboratory of "Shell" Research Ltd. for focussing our attention on halogen-sensitive detectors, Mr. D. M. Barnett for much careful experimentation, Mr. G. Blunkell for the design and construction of the stabilised power pack, and Dr. R. A. E. Galley, Director, and Mr. J. G. Reynolds, Associate Director, of the Woodstock Agricultural Research Centre, for their interest and encouragement during the course of this work.

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The Determination of Tin in Beer

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A method is described for determining small amounts of tin in beer after wet-oxidation of the sample. The method has been made rapid and simple by making use of the advantages of subtractive cathode-ray polarography. A detection limit of 0·007 μg per ml of tin in beer has been obtained.

THE practice of supplying beer in tinned cans has increased greatly during the last year or two, and this has led to a demand for the determination of tin in beer samples. It has been reported that as little as 0·02 p.p.m. of tin can give rise to hazes in beer,[1,2,3] and with tin concentrations greater than 0·1 p.p.m. the degree of turbidity is aesthetically intolerable. It is necessary to use a method of high sensitivity for determining less than 0·1 p.p.m. of tin, and, because it is likely that several other metals will be present at similar concentration levels, the method should also be specific. Most of the colorimetric methods for determining tin lack the required sensitivity, and are prone to interference. For example, according to Sandell[4] no colour is produced with dithiol when less than 0·3 μg of tin per ml is present, and bismuth, copper, nickel, cobalt, mercury, silver, lead, cadmium and some other metals interfere. Polarography has been used for determining tin in foods; Godar and Alexander[5] determined tin after co-precipitation with aluminium hydroxide, and Markland and Shenton[6] introduced a simpler method involving no separation. Condliffe and Skrimshire[7] used a single cell cathode-ray polarograph for determining tin in various foodstuffs, but they did not report any results lower than 20 to 30 p.p.m. In general, the permissible tin content of foods is quite high, and it is only because of its deleterious effect on the appearance of the product that determinations of such high sensitivity are required for beer. Recent developments in polarography have led to the development of instruments with much higher sensitivity, and it was considered likely that the use of subtractive polarography[8] would give a lower detection limit than has previously been attainable.

EXPERIMENTAL

The detection limit for tin in the selected base electrolyte (5 N hydrochloric acid) was determined by measuring the peak obtained for tin on ten solutions containing 0·02 μg of tin per ml. The standard deviation, σ, obtained corresponded to 0·0028 μg per ml, which on a 2σ basis corresponds to a detection limit of 0·0056 μg per ml. These figures were obtained by subtracting base-line effects, etc., 5 N hydrochloric acid being used in the reference cell of the polarograph. The improvement in sensitivity amply justifies the slightly increased complexity of the procedure. The improvement is illustrated in Figs. 1 to 4.

TABLE I

RECOVERY OF ADDED TIN

Sample	Tin added, μg per ml	Tin found, μg per ml	Tin recovered, μg per ml
Light ale (bottled) ..	Nil	Nil	—
	0·020	0·019	0·019
	0·050	0·045	0·045
	0·10	0·10	0·10
	0·15	0·13	0·13
	0·20	0·19	0·19
Lager (canned)	Nil	0·062*	—
	0·050	0·11	0·048
	0·15	0·20	0·14
Guinness (bottled) ..	Nil	Nil	—
	0·020	0·020	0·020
	0·050	0·045	0·045
	0·10	0·098	0·098
	0·50	0·49	0·49

*Mean of 3 determinations

The wet-oxidation of beer samples was next investigated, and it was found that digestion with nitric, perchloric and sulphuric acids by the proposed procedure gave satisfactory destruction of the sample. Recoveries of added tin were investigated by making additions of stannic sulphate solutions to beer samples. The additions made, and the recoveries obtained, are shown in Table I. It was considered that these results indicated satisfactory recovery, and the method was applied to several beer samples. The results obtained on these samples are shown in Table II.

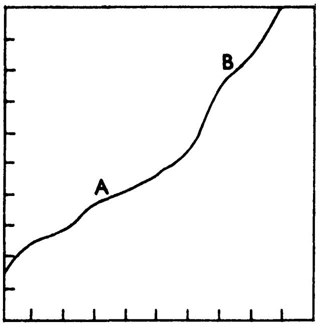

Fig. 1. Polarogram of beer containing: A, 0·031 μg of tin per ml; B, 0·18 μg of cadmium per ml. (Sensitivity $\frac{1}{25}$)

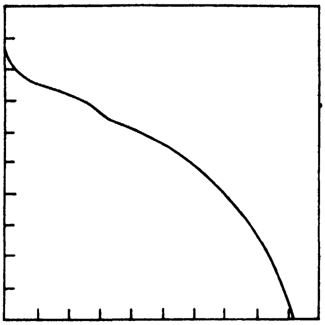

Fig. 2. Polarogram of 5 N hydrochloric acid base electrolyte, cell (II). (Sensitivity $\frac{1}{25}$)

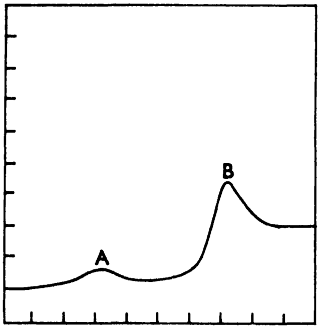

Fig. 3. Subtractive polarogram of beer containing: A, 0·031 μg of tin per ml; B, 0·18 μg of cadmium per ml. (Sensitivity $\frac{1}{10}$)

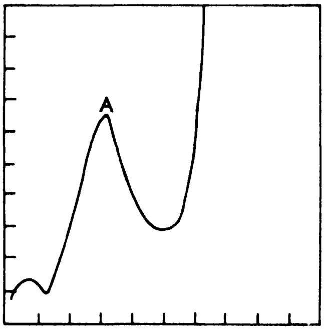

Fig. 4. Subtractive polarogram of beer containing: A, 0·031 μg of tin per ml. (Maximum sensitivity)

TABLE II

RESULTS OBTAINED ON VARIOUS BEER SAMPLES

Sample	Tin, μg per ml
Guinness (bottled)	<0·01, <0·01, <0·01
Light ale (bottled)	<0·01, <0·01, <0·01
Swiss lager (bottled)	<0·01, <0·01
Same Swiss lager (canned)	0·066, 0·058
Same Swiss lager (aluminium can) ..	0·044, 0·040
Swiss lager (canned)	0·10, 0·11
Swiss lager (bottled)	0·031, 0·026
Dutch lager (canned)	0·056, 0·061, 0·069

It is probable that the limit of detection of the whole analytical procedure will be at a higher concentration than that found for pure tin solutions, and this was found to be so in practice. It has been suggested[9] that the standard deviation of the blank value is likely to be the limiting factor in an analytical procedure, and the standard deviation of the blank value was therefore determined by analysing eight blank solutions over a period of 4 days; the determinations were carried out by a single analyst. The standard deviation was found to be 0·009 μg of tin per ml in the cell, and by using the procedure described below this corresponds to 0·0036 μg per ml in the beer sample. The limit of detection of the method on a 2σ basis is considered to be about 0·007 μg per ml, and samples in our laboratory which give results indistinguishable from the blank value are reported as <0·01 μg per ml.

Stannic chloride has a boiling-point of 114° C,[10] and use has been made of its volatility as a method of separation.[11] It is likely, therefore, that if samples rich in chloride are wet-ashed some tin may be lost by volatilisation; some of the samples investigated in the early part of this work gave low results when charred with nitric acid alone. It was found that when the sample was first charred with sulphuric acid, as in the procedure described below, satisfactory results were obtained.

In the 5 N hydrochloric acid base electrolyte used for determinating stannic tin, the only likely interfering substance will be lead. As the lead content of beer is usually low, the bulk of the lead present will probably come from the reagents, and will therefore be deducted with the blank value. Several samples had been investigated by making separate determinations of lead in a base electrolyte buffered to pH 4·6, at which tin is non-reducible, showed that the correction to be made for lead content was, in every experiment, negligible. No such correction is therefore incorporated in the proposed procedure, although if desired it can be made by using a separate determination of lead on a second portion of the solution. The statutory limit for the lead content of beer is 0·5 p.p.m., but this limit is seldom reached in practice. If substantial amounts of lead are present, then the shape of the reduction peak obtained will be altered; the tin^{IV} reduction peak is very much sharper than the lead^{II} reduction peak, and little experience is necessary before the slightly more blunt combined tin - lead peak can be readily differentiated from a pure tin peak. Interference from arsenic is prevented, as it will have been oxidised to arsenic^{V}, and the presence of germanium^{II}, niobium, rhenium or thallium is unlikely. These are the only other elements that will give a reduction peak at −0·5 volts.

METHOD

REAGENTS—

Perchloric acid, sp.gr. 1·54—Lead-free for foodstuffs analysis.
Nitric acid, sp.gr. 1·42—Lead-free for foodstuffs analysis.
Sulphuric acid, sp.gr. 1·84—Lead-free for foodstuffs analysis.
Hydrochloric acid, sp.gr. 1·18—Lead-free for foodstuffs analysis.

APPARATUS—

All glassware including cover glasses should be of Pyrex or similar glass to minimise the lead blank values. The polarograph used for this work was the Southern Analytical A1660 Differential Cathode-Ray Polarograph.

PROCEDURE—

Pour the well-shaken beer sample into an open beaker, and leave for about 20 minutes to liberate most of the dissolved gases. If this precaution is not taken, it is difficult to measure the sample by means of a pipette.

Transfer by means of a pipette 25 ml of the sample to a 100-ml beaker, cover the beaker, and evaporate almost to dryness; it will be necessary to heat gently until frothing ceases. Add 2 ml of sulphuric acid, and char the sample by gently heating. Allow to cool somewhat, and add 5 ml of nitric acid. Evaporate until fumes of sulphur trioxide appear; the sample should char again. Allow to cool, and add a further 5 ml of nitric acid and then 3 ml of perchloric acid. Evaporate gently until fumes of perchloric acid appear, and then until fumes of sulphur trioxide appear. Remove the cover from the beaker, and evaporate the solution to dryness. Allow the residue to cool, add 5 ml of hydrochloric acid, cover the beaker, and warm gently to dissolve the residue. Transfer the solution to a 10-ml calibrated flask and make up to the mark with distilled water (see Note 1). Transfer a portion of this solution to a polarograph cell, remove dissolved oxygen with a stream of nitrogen, and measure the tin peak at −0·5 volts *versus* the mercury pool anode (see Note 2). It was found preferable to use 5 N hydrochloric acid in the reference cell of the instrument, for highest sensitivity, and to measure the difference wave obtained. At least two reagent blank solutions should be processed, omitting the sample of beer, and these blank solutions should also be measured against the 5 N hydrochloric acid reference solution.

NOTES—

1. For high sensitivity polarography it has been reported[12,13] that de-ionised water can cause difficulties, and the use of distilled water for base electrolytes is recommended.

2. The polarograms obtained should contain two or perhaps three peaks, caused by copper at —0·25 volt, combined tin - lead at —0·5 volt and possibly cadmium at —0·7 volt. If this simple pattern is not obtained, or if large concentrations of copper or tin appear to be present, the wet-oxidation is probably incomplete and the determination should be repeated. It is necessary for the final evaporation to be carried out slowly, so that perchloric acid condenses on the walls of the beaker. The technique described has been successfully used on samples of lager, light ale and Guinness.

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The Polarographic Determination of Lead in Steels and Copper - Zinc Alloys

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Lead is separated from interfering elements by an ion-exchange medium. The difference in electrical conductivity between the lead-free and lead-containing eluates is used as a variable to effect the separation by automatic control. The separation technique and polarographic determination are described. Coefficients of variation for lead contents of 0·01 per cent. and 0·3 per cent. are ±4·25 per cent. and ±1·07 per cent., respectively.

THE object of this work was the development of a simple method for determining low concentrations of lead (about 0·01 per cent.) in steels and copper - zinc alloys. It was desirable that the method developed should use instrumentation already available and be sufficiently flexible to apply to both plain carbon and alloyed steels.

Published papers[1 to 7] did not appear to meet all these requirements, but Carson[8] has described an ion-exchange separation of lead and zinc from cobalt, in 2 M hydrochloric acid solution, and has suggested that this should be applicable to lead in steel. The work of Kraus and Nelson,[9] with anion-exchange resins, suggested that separation of lead from iron and most of the usual alloying elements in steel, and also copper, should be feasible at hydrochloric acid concentrations of either 9 M or 2 M.

Separation in hydrochloric acid solutions by anion exchange was attractive because this medium could act as solvent, eluent and polarographic base electrolyte. Further, if 2 M acid was chosen as the initial eluent, the change in electrical conductivity caused by the dilution of the eluting acid could be used to operate an automatic system for isolating the fraction containing the lead.

In view of these considerations, the method chosen involved an anion-exchange separation and determination of lead by linear-sweep cathode-ray polarography.

EXPERIMENTAL

Initial experiments using British Chemical Standard No. 212 (0·3 per cent. leaded steel) indicated that the ideal volumes and concentrations of eluting acid were 50 ml of 2 M, 100 ml of M and 300 ml of 0·005 M hydrochloric acid. The separation of iron and lead was further improved by allowing some degree of mixing between each concentration before entering the column.

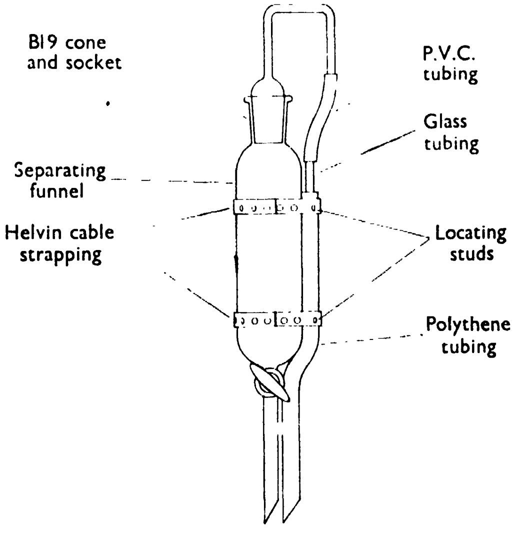

Fig. 1. Feeder funnels (350-ml and 100-ml capacity)

To introduce a degree of automation, a 100-ml burette, three-quarters filled with an analytical-reagent grade chloride-form strongly basic anion-exchange resin (De-Acidite FF), was fitted with a glass cup and a form of self regulating feed for the eluting acid (see Fig. 1). A conductivity cell was fitted at the base of the column so that the eluate passed through this cell and an anti-siphon tube to a two-way servo tap. This consisted of a spring-operated three-way tap with a relay (G.P.O. 2000 type) used as an electromagnetic catch. When the relay was energised, a spring-loaded arm was released and this rotated the tap-key, diverting the eluate from waste to sample beaker. The tap was re-set manually between separations. The tap relay was controlled by the sensitive relay (in our case a recorder), which was coupled to the output of the conductivity bridge, so that when the conductivity of the eluate was the same as the bridge setting, the tap relay was energised (see Fig. 2).

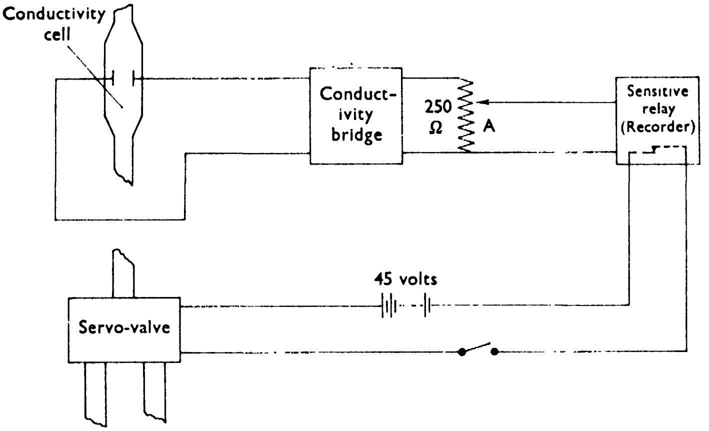

Fig. 2. Block diagram of electrical circuit Description: for the sensitive relay, a 50-mV electronic recorder with a microswitch on the pen-travel was used. By increasing the resistance of potentiometer A to 500 ohms, a sensitive electro-magnetic relay can be used in place of the recorder, which has a 50 μA operating current on a 2000 ohm coil

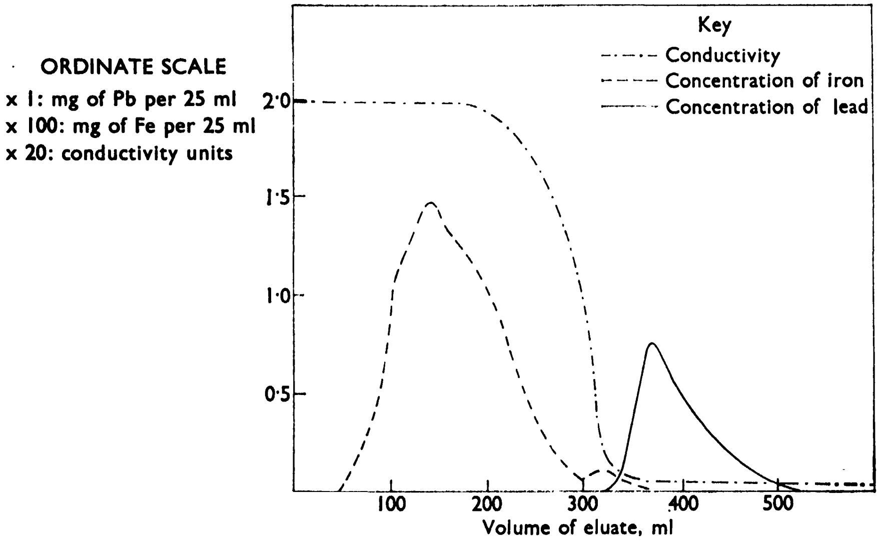

Fig. 3. Graph relating electrical conductivity, lead concentration and iron concentration to volume of eluate

The apparatus proved to be reliable and the efficiency of the separation is shown in Fig. 3. For this graph, iron was determined colorimetrically and the lead estimated by using a K 1000 polarograph.

METHOD

All reagents used should be of analytical-reagent grade.

STEELS—

Dissolve 1 g of sample in 25 ml of diluted hydrochloric acid (1 + 1) with the minimum of heating, and add nitric acid, sp.gr. 1·42, dropwise until no further reaction occurs. Boil the solution free from nitrous fumes, cool, filter off any residue and dilute to 50 ml with water.

Regenerate the column with 350 ml of 2 M hydrochloric acid and introduce the sample solution into the cup at the top of the column. Set the servo tap to waste, and adjust the flow-rate through the column to 8 to 10 ml per minute. Add a further 50 ml of 2 M hydrochloric acid to the cup and fill the two eluant feeder funnels with 100 ml of M and 350 ml of 0·005 M hydrochloric acid. Open the taps on the feeder funnels, and switch on the conductivity bridge and associated circuits.

When the conductivity of the eluate falls to the bridge setting (see "Bridge Setting"), the servo tap diverts the eluate to a sample-collector and the lead-containing fraction of the eluate is collected. Evaporate this solution to approximately 30 ml, add 5 ml of 3 per cent. w/v hydroxylammonium chloride solution while still hot, and boil the solution to complete the reduction of iron. Cool, dilute with water to 50 ml, and record the peak-height on a K1000 polarograph at −0·45 volt, against a standard calomel electrode, using an initial potential of −0·35 volts. A blank experiment on all reagents should be performed, and the sample peak-height corrected for the blank value. Convert to percentage lead by reference to a calibration graph.

COPPER - ZINC ALLOYS—

For lead contents between 0 and 1 per cent. dissolve 1 g of sample in 30 ml of diluted hydrochloric acid (1 + 1) and 1 ml of nitric acid, sp.gr. 1·42, with the minimum of heating, and proceed as for steels.

CALIBRATION—

The calibration graph was prepared by analysing 1 g quantities of Specpure iron, to which were added 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of standard lead solution (1·5980 g of Specpure lead nitrate, $Pb(NO_3)_2$, diluted to 1 litre). The quoted lead additions cover a range from 0 to 0·5 per cent. for a 1 g sample.

BRIDGE SETTING—

Obtain a graph relating conductivity, and iron and lead concentrations to volume of eluate (see Fig. 3) by performing an experiment on British Chemical Standard No. 212 (0·3 per cent. leaded steel) and analysing the eluate in 25-ml fractions. From this graph deduce the conductivity for the optimum separation of iron and lead. Set the conductivity bridge at this figure.

APPLICATION TO CARBON STEEL

Six 1-g samples of Specpure iron (Johnson Matthey & Co. Ltd.) containing 0·01 per cent. (added) lead impurity, together with a blank sample, were analysed as described in the method.

The results obtained after correction for the lead content of the blank sample showed that the mean value for the recovery of lead was 102 per cent., the standard deviation was $\pm 4{\cdot}34 \times 10^{-4}$ and the coefficient of variation was ±4·25 per cent.

Suitable standard steel samples with a lead content between 0·01 per cent. and 0·05 per cent. were not available. Consequently, we tested the reliability of the method with an actual steel, British Chemical Standard No. 212 (leaded steel, certificate value 0·28 per cent. of lead). Six 1-g samples of this steel, together with a blank sample were analysed. Results showed that the mean value for the lead content was 0·282 per cent., the standard deviation was $\pm 3{\cdot}03 \times 10^{-3}$ and the coefficient of variation was ±1·07 per cent. When British Chemical Standard No. 212/1 (leaded steel, certificate value 0·22 per cent. of lead) became available, it was also examined. Results showed that the mean value for the lead content was 0·215 per cent., the standard deviation was $\pm 3{\cdot}29 \times 10^{-3}$ and the coefficient of variation was ±1·53 per cent.

The results show that in carbon steels, lead contents of 0·01 per cent. to 0·3 per cent. can be determined with reasonable accuracy.

APPLICATION TO ALLOY STEELS AND COPPER-ZINC ALLOYS—

Kraus and Nelson[9] showed that most interferences are eliminated by the column separation described, which should leave only zinc^{II}, cadmium^{II}, indium^{III}, antimony^{III}, and, possibly, molybdenum^{VI} and tin^{IV} ions in the lead fraction. In polarographic analysis with a dilute hydrochloric acid supporting electrolyte, zinc and cadmium have sufficiently negative half-wave potentials to prevent their interference with the determination of lead. In fact, zinc and cadmium in the lead-containing eluate could be quantitatively determined polarographically. It was most unlikely that indium would be present in steels or copper-zinc alloys, but if present probably would not interfere. Antimony would give a polarographic wave only in strongly acid conditions, but this wave is well removed from that of lead. The presence of molybdenum was shown to have no effect on the recovery of lead.

Any tin present in the lead-bearing eluate could seriously interfere with the polarographic determination as the waves of tin and lead in chloride media coalesce.

Small amounts of tin tended to remain on the column and were eluted with the lead in the next sample. This problem was solved for 1 g samples containing 0·2 per cent. of tin by using at least 350 ml of regenerant acid between separations (see Table I).

TABLE I

DETERMINATION OF LEAD IN BRITISH CHEMICAL STANDARD NO. 275 STEEL CONTAINING ADDED TIN

(Certificate value: 0·04 per cent. of tin, 0·005 per cent. of lead)

Tin added, per cent.	Total tin, per cent.	Lead found, per cent.	Recovery, per cent.
Nil	0·04	$0{\cdot}004_9$	98
0·20	0·24	$0{\cdot}005_2$	104
Nil	0·04	$0{\cdot}004_9$	98
Nil	0·04	$0{\cdot}005_1$	102
0·20	0·24	$0{\cdot}005_4$	108
Nil	0·04	$0{\cdot}005_0$	100

To determine the effect of tin in a copper - zinc alloy on the recovery of up to 0·2 per cent. of lead, we made a 60+40 copper - zinc alloy and added 0·2 per cent. of lead, and also we made a 60+40 copper - zinc alloy and added 0·2 per cent. of tin.

In one series of experiments (6 samples) we added 0·01 per cent. of lead, and found a recovery of between 94 per cent. and 105 per cent., with a mean value of 100 per cent. In the second series of experiments (4 samples), we added 0·2 per cent. of lead, and found a recovery of between 95 per cent. and 100 per cent., with a mean value of 97·5 per cent.

These experiments showed that lead, present to the extent of 0·01 per cent., could be determined accurately in carbon and alloy steels, and in copper - zinc alloys, provided that the tin content did not exceed 0·2 per cent.

CONCLUSIONS

The method has proved reliable for the routine determination of lead contents between 0·005 per cent. and 1 per cent. in a wide variety of steels and copper - zinc alloys, the only limitation being that the tin content for a 1-g sample should not exceed 0·2 per cent.

We thank the Director, A.W.R.E., Aldermaston, for permission to publish this report.

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SHORT PAPERS

The Determination of Glucose in the Presence of Maltose and Isomaltose by a Stable, Specific Enzymic Reagent

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DURING investigations on the degradation of starch by fungal enzymes, a simple and accurate micro method was required for determining glucose in the presence of other reaction products. The Barfoed reagent of Tauber and Kleiner[1] is specific for glucose in the presence of maltose, but reproducible results are obtained only if conditions are carefully standardised; even then, the reagent responds to isomaltose as well as to glucose.

Other methods were therefore investigated, and of these a modification of the glucose oxidase procedure was found most satisfactory. All the glucose oxidase enzyme preparations tested, unless prepared in tris buffer as described by Dahlqvist,[2] were found to react with maltose and isomaltose as well as with glucose. This enzyme reagent was further tested, and found to be highly specific for glucose, though it suffered from the disadvantage of relative instability. The colour developed was linearly related to glucose concentration only up to 50 μg of glucose per ml of solution. The addition of up to 40 per cent. w/v glycerol, as recommended by Washko and Rice,[3] was found to increase the stability considerably, without altering the specificity of the Dahlqvist enzyme reagent. A number of colour-developing reagents were tested; of these *o*-dianisidine hydrochloride, at the specified concentration was found to be the most sensitive, particularly in presence of 5 N sulphuric acid.[3] These modifications have been found to increase the life of the enzyme reagent from a few days to over three months at 4° C, and to increase the linear response up to 100 μg of glucose per ml of test solution.

TABLE I

ESTIMATION OF GLUCOSE IN THE PRESENCE OF MALTOSE OR ISOMALTOSE BY GLUCOSE OXIDASE AND BARFOED REAGENT

Mixture contained— 50 μg glucose, *plus*	Glucose found (μg) by using— Glucose oxidase in phosphate	Glucose oxidase in tris buffer	Barfoed reagent
500 μg maltose	65	50	60
500 μg isomaltose	53·5	50	103

METHOD

REAGENTS—

Glucose oxidase, 10 mg—C. F. Boehringer und Soehne, Mannheim, Germany.

Horseradish peroxidase, 1 mg—Worthington Biochemical Corporation.

o-*Dianisidine hydrochloride, 10 mg*—Prepared from *o*-dianisidine.

Tris glycerol buffer, pH 7·0—Prepare by dissolving 61 g tris-(hydroxymethyl)aminomethane in 85 ml of 5 N hydrochloric acid, dilute the solution to 1 litre with water and add 660 ml of analytical-reagent grade glycerol. Adjust the pH to 7·0 if necessary.

PROCEDURE—

Add 2 ml of the reagent to 1 ml of solution containing 0 to 100 μg of glucose. Mix the solutions and incubate them at 25° C for 1 hour. (Include standard glucose solutions.) After incubation, add 4 ml of 5 N sulphuric acid, mix, and measure the optical density at 525 mμ. (The colour is stable for at least 12 hours.) Prepare standards from a stock solution containing 2 mg of analytical-reagent grade glucose in saturated aqueous sorbic acid. This solution keeps indefinitely. Dilute stock solution to 1 + 20 for a working standard, and use this standard for blank determinations when performing analyses.

RESULTS

The effect of maltose and isomaltose on the determination of glucose by glucose oxidase (with phosphate or tris buffer) and Barfoed reagent is shown in Table I.

Fig. 1 shows the response curves for the various reagents.

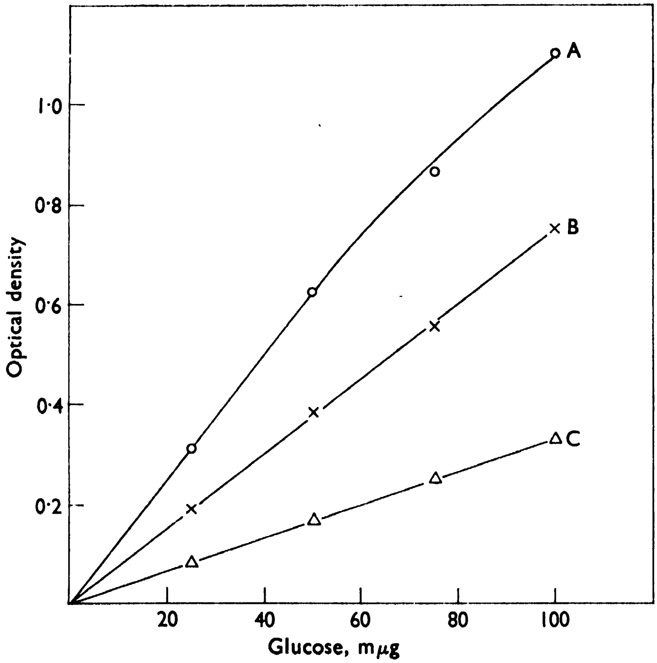

Fig. 1. Calibration curves for glucose: curve A, Dahlqvist reagent; curve B, modified reagent; curve C, Glucostat reagent

DISCUSSION

As shown in Table I, the Barfoed copper reagent and the Glucostat glucose oxidase reagent, which contains phosphate buffer, are not specific for glucose in the presence of maltose and isomaltose. When the phosphate buffer is replaced by tris buffer at pH 7, the maltose and isomaltose activities are inhibited, and the reagent is specific for glucose. A further disadvantage of the Glucostat reagent is its low sensitivity compared with either Dahlqvist or the proposed reagent (see Fig. 1) both of which are specific for glucose. The proposed reagent although less sensitive than that of Dahlqvist, has a greater range and the additional advantage of being completely stable for at least six months.

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Improved Paper-chromatographic Separation of Sugar Phosphates by using Borate-impregnated Paper

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THE borate-complexing technique for separating sugar phosphates has been successfully used in ion-exchange chromatography[1,2] and paper electrophoresis.[3,4] It has met with limited application in paper chromatography.[5,6,7] Cohen and Scott[5] used boric acid in neutral ethanol to achieve a sharp separation between aldopentose-5- and 3-phosphates, but the separation among the individual 5-phosphates or the 3-phosphates was not satisfactory. The solvent system failed to effect a satisfactory resolution among the hexose-monophosphates. According to Isherwood,[6] incorporation of boric acid in the alkaline n-propanol system readily effected a separation between glucose-1-phosphate and 6-phosphate. Baron and Brown[7] achieved success in separating ribose-2-, 3- and 5-phosphates by introducing boric acid in the propanol - ammonia solution system.

A scheme of paper-chromatographic separation of a mixture, containing essentially hexose-phosphates, by using borate-containing solvents has not been proposed. Nor has, to our knowledge, borate-impregnated paper been used in the separation. A simple and rapid method, in which borate-impregnated paper is used, has been developed by us, and this permits a fairly sharp separation of the components from a mixture of orthophosphate and five sugar phosphates.

METHOD

PROCEDURE—

The samples of sugar phosphate in the form of sodium, potassium, or barium salts, obtained from recognised commercial sources, were de-cationised with a cation-exchange resin, in the hydrogen form, and the solutions neutralised to pH 7 with 0·1 M ammonia solution. The basic borate-free solvent system consisted of 60 volumes of t-butanol (this was superior to n-propanol[6]), 30 volumes of ammonia solution (sp.gr. 0·88) and 10 volumes of water. In the borate-containing solvent system, water was replaced by a solution of sodium tetraborate to give a final concentration of 0·01 M borate. Whatman No. 1 filter-paper strips (12 cm × 38 cm) were used, either without any treatment or after being dipped in borate solution (0·001 M to 0·01 M) and dried at room temperature. Where pre-complexing of esters with borate before application to paper was desired, stock solutions of the phosphate esters were made in 0·01 M borate solution. Portions of the solutions, containing 0·1 to 0·2 μmoles of sugar phosphates, were applied to the paper and dried. One-dimensional descending-solvent chromatograms were developed for about 24 hours at room temperature, the solvent being allowed to overflow and drip from the serrated edge of the paper. The air-dried chromatograms were treated with the spray reagent of Runeckles and Krotkov.[8]

RESULTS AND DISCUSSION

Spots were usually round or slightly pear-shaped. The migrations of various esters have been calculated as R_P values. This is the distance from the base line to the centre of each phosphate ester spot, as related to the corresponding distance of the orthophosphate spot, the latter being taken as 100 for convenience. Typical R_P values, obtained for various sugar phosphates under the influence of borate ions, are given in Table I.

There was little separation between the various sugar monophosphates in the non-borate system. The use of borate effected an alteration in the migratory behaviour, and the degree of alteration depended on several factors. The glucose-1-phosphate spot invariably moved faster than the other phosphates under all the conditions used, showing that this ester remained uncomplexed with borate.[1] The uncomplexed orthophosphate followed the glucose-1-phosphate under identical conditions. Glucose-1- and 6-phosphates separated from each other after merely introducing borate into the solvent system. This confirmed the observations of Isherwood,[6] but fructose-6-phosphate and ribose-5-phosphate were not separated from glucose-6-phosphate. The use of borate pre-complexed esters did not offer any significant advantage. Considerable improvement in resolution resulted when borate-impregnated paper was used instead of untreated paper. The concentration of borate critically affected the optimum separation. When 0·01 M borate was present, fructose-6-phosphate separated from the other two non-glucosidically phosphorylated monoesters, but ribose-5-phosphate and glucose-6-phosphate did not separate sharply. When the concentration of borate was halved (0·005 M), a separation between glucose-6-phosphate and ribose-5-phosphate tended to occur. At 0·001 M borate-ion concentration, glucose-6-phosphate moved as fast as orthophosphate. Such behaviour is understandable if one assumes, as did Khym and Cohn,[1] that glucose-6-phosphate is capable of dual behaviour, changing from the highly complexed furanose form to the less complexed pyranose form at lower borate-ion concentration. Excellent separation of all the components was found to occur when 0·002 M borate was used for paper impregnation.

TABLE I

R_P VALUES OF SUGAR PHOSPHATES ON PAPER CHROMATOGRAMS UNDER THE INFLUENCE OF BORATE IONS

(R_P value for orthophosphate = 100)

System	R_P values for				
	Glucose-1-phosphate	Glucose-6-phosphate	Fructose-6-phosphate	Fructose-1,6-diphosphate	Ribose-5-phosphate
Non-borate system, control ..	120	116	114	59	114
Borate system, untreated paper:					
Irrigating solvent containing 0·01 M borate	121	106	111	65	107
Esters pre-complexed with 0·01 M borate	118	87	83	41	70
Borate-impregnated paper:					
0·01 M	117	58	83	40	63
0·005 M	125	61	79	31	55
0·004 M	127	65	63	27	50
0·003 M	121	66	73	32	47
0·002 M	125	86	76	31	41
0·001 M	140	97	86	40	62
Combination of techniques:					
Esters pre-complexed with 0·01 M borate, on 0·01 M borate-impregnated paper	114	61	79	40	61
Irrigating solvent containing 0·01 M borate, on 0·005 M borate-impregnated paper ..	108	64	89	58	69

Combinations of these techniques, for example, by using pre-complexed esters with impregnated paper, or borate-containing irrigating solvent with impregnated paper, did not satisfactorily separate all the components.

The results reported above for single components were also applicable when the components were present in a mixture. The present method represents a definite improvement on our earlier chromatographic method,[9] in which borate ions were not used.

One of us (D.P.A.) thanks the Scientific Research Committee, U.P., Allahabad, and the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

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A Field Test for Aniline Vapour in Air

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THE test recommended by H.M. Factory Inspectorate[1] for detecting and estimating aniline vapour in air involves the collection of the aniline in dilute hydrochloric acid, boiling this solution with bleaching powder and finally developing a permanent blue colour by reaction with ammoniacal phenol solution. The intensity of the colour is then estimated by comparison with liquid standards prepared from a blue dye or with Lovibond permanent glass standards.

In a recent investigation in this laboratory, it became necessary to determine low concentrations of aniline in air. The official method was used, but standards were prepared from carefully purified aniline hydrochloride rather than the blue dye. During the preparation of these standards, it was found that the intensity of the colour developed largely depended on the rate at which the solution was brought to the boil, and the length of time for which it was boiled. The variations in colour intensity were sufficient to vitiate the accuracy of the method. Even under controlled conditions, the resulting colours, when arranged in order of increasing intensity, frequently placed the standards in an incorrect order. We were unable to find any convenient means of obtaining reliably reproducible colours. Since the occurrence of such errors would not be apparent when the recommended types of colour standard were used, an alternative method, avoiding the necessity for boiling, appeared desirable for use in the field.

The method recently suggested by Hands[2] as a field test for nitrobenzene vapour in air was considered suitable for adaptation since it involved initial reduction of the nitrobenzene to aniline which was then estimated by a method very similar to the one described by Strafford, Strouts and Stubbings.[3] That part of the test involving the diazotisation and coupling of aniline with disodium 2-naphthol-3,6-disulphonate (R-salt) was shown by Hands to be superior to various alternatives under the conditions of the test, and is sufficiently sensitive for determining hazardous concentrations of aniline. Other reagents, described by Daniel,[4] give a greater sensitivity than is required here.

The adoption of Hands' method for estimating aniline has the great advantage that the same apparatus and reagents would be used in field tests for nitrobenzene, aniline and many other aromatic amines. Owing to the lower toxicity of aniline,[5] compared with nitrobenzene, a different range of standards must be prepared if the aniline test is to cover that concentration range that is of industrial interest. Alternatively, the original liquid or glass standards[2] could be used with a proportionately smaller air sample, and a correction made for the increased sampling efficiency. The bubbler collection efficiency in this instance proved to be 100 per cent., so that there was no need to apply a correction factor in the preparation of standards.

METHOD

APPARATUS—

Bubbler—This should be an all-glass construction. The solvent container should be 9 cm deep and 1·3 cm in diameter, with a centrally blown bulb of diameter 2·5 cm. The internal diameter of the delivery tube should be 2 mm.

Pump—A metal hand-pump of 120 ml capacity or other form of suction device[6,7] capable of giving a rate of sampling of 1500 ml per minute should be used.

Trap—A suitable trap consists of a wide-necked bottle of about 100 ml capacity, fitted with a 2-hole bung and delivery tubes and connected between the bubbler and the pump.

Comparator tubes—13 mm × 8 cm standard tubes or rectangular cells.

Pipettes—2- and 5-ml dropping pipettes with rubber teats.

REAGENTS—

All chemicals used should be of analytical-reagent grade.

Hydrochloric acid—Dilute 5 ml of concentrated hydrochloric acid, sp.gr. 1·18, to 100 ml with water.

Sodium nitrite solution—Dissolve 3·5 g of sodium nitrite in 100 ml of water. This solution should not be kept for more than one month.

Sodium carbonate solution—Dissolve 10 g of anhydrous sodium carbonate in 100 ml of water.

R-salt solution—Dissolve 0·8 g of the purified sodium salt of 2-naphthol-3,6-disulphonic acid in 100 ml of boiling water, adjust the pH to between 7·5 and 8·5 by adding M sodium carbonate, cool to room temperature and filter. This reagent should be stored in the dark, and should not be kept for more than one month.

Ammonia solution—Dilute 20 ml of ammonia solution, sp.gr. 0·88, to 100 ml with water.

COLOUR STANDARDS—

Dissolve 1·000 g of potassium dichromate in 1 litre of water; this is solution A. Dissolve 70·26 g of cobalt sulphate septahydrate in 1 litre of water; this is solution B. Prepare the standards by placing the amounts of water and solutions A and B shown in Table I into 13-mm cells or comparator tubes.

TABLE I

COMPOSITION OF COLOUR STANDARDS

Aniline concentration, p.p.m. v/v	Solution A, ml	Solution B, ml	Water, ml
1	2·0	1·8	23
2·5	4·0	6·0	19
5	5·2	8	16
10	10	10	4

PROCEDURE—

Place 5 ml of the hydrochloric acid solution in a clean, dry bubbler and connect this to the pump via the trap. Take a 6-litre sample of the air under test at the rate of 1·5 litres per minute. (If the hand pump is used, 50 strokes at a rate of 5 seconds per exhaust stroke are required.) Detach the bubbler. Transfer its contents to a clean, dry 10-ml calibrated comparator tube. Wash out the bubbler with two successive 2-ml portions of the hydrochloric acid solution, transferring the washings to the calibrated tube. Make up the volume in the tube to 10 ml with the hydrochloric acid solution.

By means of the teat pipette, transfer 5 ml of this solution to another clean, dry 10-ml comparator tube, add 0·5 ml of the sodium nitrite solution, mix and allow to stand. After 2 minutes add 2 ml of the sodium carbonate solution, shake and then add 0·5 ml of the R-salt solution. Shake again to mix thoroughly and add 2 ml of the ammonia solution, shake and compare the colour of the solution with those of the colour standards.

NOTES—

1. Aniline concentrations outside the range 1 to 10 p.p.m. can be determined by varying the size of the air sample. The stated rate of sampling must not be exceeded if the collection efficiency of the bubblers is to be maintained.
2. The liquid colour-standards were prepared by matching the colour produced from standard concentrations of aniline hydrochloride in the hydrochloric acid solution against those of mixtures of the inorganic salt solutions.
3. The bubbler collection efficiency was checked by passing air containing aniline vapour through two bubblers in series. When the bubblers were operated under the stated conditions, no aniline hydrochloride could be detected in the second bubbler, either by the proposed colorimetric method or by ultraviolet absorption.

We thank Mr. R. A. Bragger for assistance with the experimental work.

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The Use of a Constricted Rubber Bulb for Obtaining an Average Sample of an Atmosphere During a Working Period

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THE slow-sampling apparatus was primarily designed to draw 120 ml of air through a trichloroethylene indicator tube,[1] at a constant rate over a period of 8 hours, so that the indication obtained represents the average concentration of the contaminant during this period.

The method depends on the fact that the pressure within a deflated thick rubber bulb is reasonably constant, irrespective of the amount of deflation. The vacuum of the bulbs we use (Siebe Gorman sampling bulb, size 6) is equivalent to about 12 cm of mercury, and the capacity of the bulbs is about 120 ml. Against the suction head of about 12 cm of mercury, a sample flow of 15 ml per hour may be obtained by using a constriction made from 2 cm, 0·05-mm bore, tubing (polarograph capillary-tubing). We have used this type of constriction, protected from atmospheric dust by glass sinters fitted at either end. We have also found that a length of about 10 cm of electrode carbon-rod (Grade ENS, 13-mm diameter: Morgan Refractories Ltd.) is sufficiently porous to make a suitable constriction. Fig. 1 shows the slow-sampling apparatus in which the carbon-rod constriction is used. The carbon rod is fixed into the glass tube with Apiezon black wax, so that the peripheral space is completely filled with wax and the ends of the rods are clean.

The sleeve valve is constructed from a standard B24 cone and socket and this is lubricated with a heavy grease (Apiezon L is suitable). The actual sampling rate may be measured by deflating the bulb, closing the valve and then connecting the apparatus to a 1 ml graduated pipette by means of a short length of rubber tubing. The tip of the pipette is dipped in water, so that a bead of water is drawn in, and the time taken for the bead to rise through a volume in the pipette is measured.

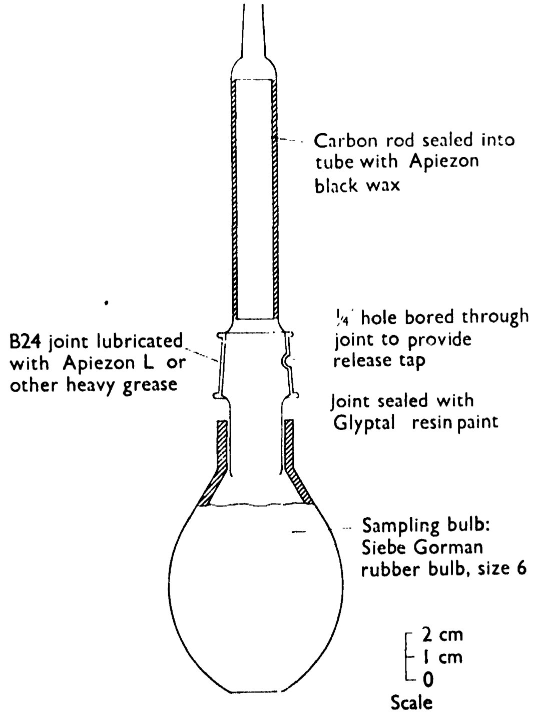
Carbon rod sealed into tube with Apiezon black wax
¼″ hole bored through joint to provide release tap
B24 joint lubricated with Apiezon L or other heavy grease
Joint sealed with Glyptal resin paint
Sampling bulb: Siebe Gorman rubber bulb, size 6
2 cm
1 cm
0
Scale

Fig 1. Constricted sampling bulb

If the sampling time is too long, it may be reduced by removing the rubber bulb and boring into the carbon by using a $\frac{5}{16}$-inch drill so that the effective length of the carbon rod is reduced. The rubber bulb is finally fixed permanently to the glass tube with Glyptal resin paint.

The calibration of the trichloroethylene indicator tube, for the normal sampling rate (4 minutes for 120 ml), remains valid at the slow sampling rate. With other indicator tubes it may be necessary to recalibrate when the sampling rate is changed.

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Titration of Weak Bases in Acetic Anhydride

BY D. B. COWELL* AND B. D. SELBY

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IN acetic anhydride the acidity of perchloric acid is markedly increased; the active "acidic" entity is probably the acetyl cation CH_3CO^+ (possibly solvated in the form $(CH_3CO)_3O^+$) rather than the solvated proton (which, in acetic acid, is in the form $CH_3COOH_2{}^+$). There is considerable evidence in support of this.[1 to 12]

Thus bases, such as amides and purines,[13,14,15] too weak to be titrated successfully in acetic acid, can be titrated potentiometically in acetic anhydride by using a modified glass - calomel electrode system. This led to the investigation of the basic properties of well-known derivatives of carbonyl compounds in this solvent. Since these are often prepared in the course of research in organic chemistry, it seemed desirable to have a method of direct titration. No satisfactory method has been reported concerning the direct titration of these derivatives as bases. The method described here is similar to that used by Wimer[16] for titrating amides, and is applied to semicarbazones, phenylhydrazones, 4-nitrophenylhydrazones and 2,4-dinitrophenylhydrazones.

METHOD

SAMPLES FOR ANALYSIS—

When commercial samples are available, the purest grades should be used without further purification, provided their melting-points agree with those quoted in reliable literature sources. Otherwise they should be purified by recrystallisation until the melting points are constant. Samples that are made should be purified likewise.

REAGENTS—

Acetic anhydride and glacial acetic acid—Good quality commercial grades.

Perchloric acid—72 per cent. aqueous solution, analytical-reagent grade.

The titrant—Prepare a 0·2 N perchloric acid in a mixture of equal parts of acetic anhydride and glacial acetic acid. Allow to stand for at least 12 hours before use. Although it may become a pale straw colour on storage it does not lose strength for at least two weeks (*cf.* Streuli[17]). Standardise it by potentiometric titration with analytical-reagent grade potassium hydrogen phthalate dissolved in glacial acetic acid, and check at frequent intervals.

APPARATUS—

Glass electrode—Cambridge wide-range type. Immersed in acetic anhydride for 12 hours before use.

Modified calomel electrode—Sleeve type; the aqueous solution being replaced by a 0·1 M solution of anhydrous lithium perchlorate in acetic anhydride as described by Wimer.[16]

Potentiometer—Cambridge pH meter, used as a potentiometer. Incorporate a Weston standard-cell into the circuit when necessary, to allow for reversed polarity of the electrodes in non-aqueous media.[18]

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PROCEDURE—

Dissolve 1 millimole of the sample (or a convenient quantity) in 40 ml of acetic anhydride using a glass-covered magnetic stirrer to assist dissolution. Cool the titration vessel to 0° C in a mixture of ice and salt (or, if rapidly soluble, dissolve at 0° C), and titrate with the 0·2 N perchloric acid. Take the usual precautions to exclude moisture. Plot a graph of e.m.f. in millivolts *versus* the volume of titrant added.

In this work it was desired to compare the basic strengths of the compounds under these conditions, and hence millimole quantities were weighed to within one milligram, and the temperature of the titration vessel was kept as constant as possible during the titrations. For substances sparingly soluble in acetic anhydride, the minimum amount of chloroform was sometimes used to dissolve them and the volume made up to 40 ml with acetic anhydride. It was found that amounts of chloroform up to 50 per cent. had little effect on the shape of the titration curve.

RESULTS

The percentage purity of the samples were calculated from the volume of titrant at the point of maximum slope of the curves.

SEMICARBAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Ethyl methyl ketone	98·7, 98·0	500
Methyl n-propyl ketone	100·5, 99·5	800
Methyl isopropyl ketone ..	97·0, 98·5	800
t-Butyl methyl ketone	101·0, 101·0	400
Mesityl oxide	98·0, 98·0	1100
2-Methylcyclohexanone	98·0, 99·0, 97·8	520
Benzophenone	98·0, 98·3	135
Benzaldehyde	98·6, 98·0	Weakly basic*
Benzalacetone	98·0, 97·0	900
Acetophenone	96·0, 95·0†	—

* Solubility insufficient to allow of one millimole to be titrated.
† Required heating to dissolve; this may account for low result.

The semicarbazones of acetone and cyclohexanone gave low results, and those of aliphatic aldehydes showed weakly basic properties and gave very low results.

PHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetophenone	99·0, 101·0	1000
Benzalacetone	98·0, 100·0, 100·2	1300
Dibenzalacetone	101·0, 101·0	200
Benzalacetophenone	99·0, 97·0	125
Benzophenone	100·0, 100·0	450
Cinnamaldehyde	100·3, 100·0	140

The aliphatic ketone and aldehyde phenylhydrazones were too unstable to be titrated successfully. The phenylhydrazones of benzaldehyde and cyclohexanone did not give stoicheiometric results.

4-NITROPHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetone	100·0, 98·5, 98·3	1250
Cyclohexanone	98·0, 98·0	1250
Ethyl methyl ketone	98·8, 99·0	900
Methyl n-propyl ketone	98·0, 100·0, 98·5	900
Methyl isopropyl ketone ..	99·4, 100·8	750
t-Butyl methyl ketone	101·0, 101·8	560
Benzalacetone	99·8, 98·0	900
Acetophenone	98·0, 98·0	300
Benzophenone	98·0, 99·0	120
Cinnamaldehyde	99·4, 99·0	140
Dibenzalacetone	Non-basic	—

The 4-nitrophenylhydrazones of acetaldehyde, formaldehyde and butyraldehyde were non-basic, while that of benzaldehyde was so weakly basic that the end-point could not be determined with accuracy.

2,4-DINITROPHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetone	100·0, 101·0	600
Cyclohexanone	98·0, 100·4	350
Ethyl methyl ketone	100·0, 98·5	550
Methyl n-propyl ketone	99·0, 98·0	500
Methyl isopropyl ketone ..	98·0, 97·0	200
2-Methylcyclohexanone	98·7, 98·0	300

The 2,4-dinitrophenylhydrazone of t-butyl methyl ketone was too weakly basic (slope 100) for the end-point to be determined accurately. The 2,4-dinitrophenylhydrazones of aromatic ketones were too sparingly soluble to be titrated and those of aliphatic aldehydes were non-basic.

OXIMES—

Oximes were non-basic in acetic anhydride with the exception of the weakly basic acetoxime but the curve was not stoicheiometric.

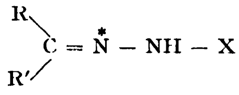
DISCUSSION

Although the method is rather limited in its application owing to the somewhat poor solvent power of acetic anhydride (with the addition, if necessary, of chloroform) the results obtained generally show a divergence of not more than 2 per cent. when millimolar amounts were used.

It was found that better and more consistent results were obtained when the titration vessel was maintained at 0° C, and the maximum slopes of the curves were usually greater than at room temperature. Further, the probability of acetylation and other reactions occurring with the solvent was reduced.

All the derivatives titrated acted as monoacidic bases in acetic anhydride and the mean maximum slopes of the curves show that the basic strength was profoundly affected by the groups attached to the original carbonyl group. Thus, in all experiments, the derivatives of aliphatic ketones were the most basic whereas, as might be expected, the presence of one or two aromatic rings markedly reduced their basic strength. The benzalacetone derivatives, however, showed remarkedly high basic strength. Aldehyde derivatives generally showed weak basic properties (slopes less than 200) or none at all. The reason for this is not understood. The method therefore cannot be recommended for the derivatives of aldehydes so far investigated.

In the general formula



$$\begin{matrix} R \\ & C = \overset{*}{N} - NH - X \\ R' \end{matrix}$$

where R and R′ are alkyl or aryl groups or H, and X is $CONH_2$, phenyl, 4-nitrophenyl or 2,4-dinitrophenyl, the substituent X had less influence on the basic strength than R and R′, even though the introduction of nitro groups into the phenylhydrazone derivatives did cause a progressive weakening of basic strength. It was therefore assumed that the nitrogen marked with an asterisk was the origin of the basic properties.

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Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume XI. Edited by DAVID GLICK. Pp. x + 442. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 110s.

This volume opens with an article by J. B. Willis of Melbourne, Australia, on the analysis of biological materials by atomic absorption spectroscopy. It will be recalled that the Fraunhofer lines in the solar spectrum are caused by the absorption of radiation from the hot centre of the sun by sodium vapour in the outer parts of its atmosphere. Emission spectra have, for a long time, been much used in chemical analysis, but atomic absorption was neglected until A. Walsh in 1953 recognised the potentialities of the method and devised suitable techniques. At temperatures between, say, 2000° and 5000° K, only a small fraction of the (vaporised) atoms is raised above the ground state, *e.g.*, for the most easily excited metal (caesium) the fraction is about 3×10^{-2} at 4000° K, and for zinc it is only about $1{\cdot}5 \times 10^{-7}$. The difference explains why flame photometry works so much better for the alkali metals than for zinc or magnesium. The vast majority of the atoms in the vapour are in the ground state and can only absorb radiation corresponding to the wavelength of the resonance lines (*e.g.*, Na, 5890, 3303 and 2853 Å; Mg, 2852 and 2026 Å; Zn, 2138 and 1590 Å; P, 1775 Å.) In atomic absorption spectroscopy, the line corresponding to the transition from the ground state to the lowest excited state is generally used. It is necessary to have a source emitting a strong sharp line radiation that is passed through the atom-charged vapour and into a monochromator. Under optimum conditions the relation between intensity of absorption and concentration is linear. The chapter has a full account of technical procedures and an interesting comparison is made between flame photometry and atomic absorption spectrophotometry. The field is an interesting one in which important developments have occurred.

E. C. Horning, W. J. A. Van der Heuvel and B. G. Creech of Houston, Texas, write on the separation and determination of steroids by gas chromatography. In the last three years the preparation of thin-film columns has allowed temperature ranges around 200° to 220° C, and retention times of 10 to 20 minutes to be used with very small samples. The method permits gas chromatography of steroids and other substances of biochemical interest. Variations in steroid separations depend on thermostable liquid phases differing in structure, and displaying selective retention effects for different functional groups. Methyl-substituted siloxane polymers (methyl silicones) are non-selective, in that separation depends mainly on differences in molecular weight or shape. A methyl fluoralkyl silicone selectively separates alcohols, ketones and esters and some polyfunctional steroids, whereas methyl phenyl siloxane polymers show selective retention for carbon - carbon unsaturation. Polyesters such as neopentyl glycol succinate and adipate are also useful. In steroid work the thermal conductivity detection systems used with ordinary thick-film columns are replaced by ionisation detection systems, based on measuring changes in conductivity of the flowing gas stream as its composition changes. One such system, devised by Lovelock, depends on ionisation by argon. The cell contains a radioactive source (α- or β-radiation), and when a high-potential is applied in the presence of radiation, metastable argon atoms are formed, but the electrical conductivity remains low. The metastable argon leads to partial ionisation of organic substances entering the cell; current flow increases and is amplified and recorded. Other detector systems are described. This article reveals how rapidly advances are being made in the field. The experiments are technically exacting and require highly trained personnel, but the analytical possibilities are great.

I. E. Bush of Birmingham discusses advances in direct scanning of paper chromatograms for quantitative estimations. Effluent and eluate methods of chromatography are compared with scanning methods. Paper chromatograms obtained under the best conditions need not be inferior to column chromatography, and scanning leads conveniently to automation. The author stresses the importance of cleanliness and likens efficient paper chromatography to aseptic surgery. After many trials he thinks that as a general rule the 'cleaning' of filter-paper is now unnecessary and he does not advise washing before use, apart perhaps from cross-washing (*i.e.*, across the long paper strips). After chromatography, the substances being studied are treated to yield light-absorbing or fluorescent products. The application of reagents must be skilfully executed in quantitative work, and detailed instructions for preparing, drying and stabilising the paper strip must be followed.

Bush goes on to discuss optical and instrumental requirements, methods of recording and the automation of scanning procedures. His existing machine CASSANDRA (Chromatogram

Automatic Scanning and Digital Recording Apparatus) is a highly complicated and successful arrangement. [Incidentally, Cassandra, daughter of Priam and Hecuba, was endowed with the gift of prophecy, but it was ordained that none should believe her predictions!]

Some specific procedures for reducing steroids, 17-ketosteroids and amino acids by the ninhydrin method, etc., illustrate how productive the automatic apparatus can be made. It can serve the needs of several busy research groups without being overworked.

Morton K. Schwartz and O. Bodansky of the Sloan-Kettering Research Institute discuss automated methods for determining enzyme activity. In their introduction they mention that "certain safeguards must be taken lest automatism on the part of the research worker replace automation by the machine," and they regard "over-automation" as quite a danger.

These authors consider three stages of automation: (1) procedures in which reaction mixtures are prepared manually but enzyme activity is recorded on an instrument; (2) automated preparation of reaction mixture as well as recording of activity; (3) procedures (not yet fully developed) incorporating feedback devices and built-in computers. "The economics of handling the increased demands by the clinician for enzyme determinations and . . . for all diagnostic biochemical determinations will be burdensome unless automation in its final or third stage is realised." As examples, many assays culminate in measurement at 340 mμ of oxidation of NAD or NADP, and when recorded on a spectrophotometer this represents a stage 1 operation. The Robot Chemist made in California is a stage 2 effort, as also is the Astra enzyme assayer and the AutoAnalyser. The latter lends itself to rapid colorimetric determinations, and the authors proceed to describe a number of standard problems successfully tackled in this way.

I. J. Kopin of Bethesda, Maryland, deals with the estimation of magnitudes of alternative metabolic pathways. This is a discussion of principles, illustrated in terms of the metabolism of adrenaline and noradrenaline. The methods involve examination of urinary metabolites of endogenous and administered labelled precursors. This is a neat and convincing treatment of an important problem.

F. L. Crane and R. A. Dilley of Lafayette, Indiana, review the determination of co-enzyme Q (ubiquinone). This is an excellent article giving the essential background of the ubiquinone story with details of extraction and spectrophotometric assay procedures. Methods based on paper chromatography are also described.

R. P. Davis of New York contributes an article on the measurement of carbonic anhydrase activity. There is no standard method, but the author describes the preparation of the enzyme and discusses manometric methods and indicator methods, as well as electrometric and histochemical problems. The information given should allow investigators to choose the best method for their particular needs.

O. H. Müller of Syracuse, N.Y., contributes a substantial chapter on polarographic analysis of proteins, amino acids and other compounds by means of the Brdička reaction. It was found in 1933, by Brdička, that certain amino acids, polypeptides and proteins, dissolved in a cobalt-containing buffer of suitable pH, produce a reaction at the dropping-mercury electrode. The currents involved in the Brdička reaction are catalytic and distinct from the non-catalytic diffusion currents that form a basis for quantitative analysis by ordinary polarography. The Brdička reaction was applied earlier to the diagnosis of malignancy, but more recent work indicates that, although the test is not specific for cancer, it is nevertheless useful in biochemical analysis. (The results should be reported in terms of current density μA per sq. mm of electrode surface.) Characteristic protein double-waves are not obtained in a cobalt-free buffered solution of serum. The Brdička effect is due to a catalytic reduction of hydrogen ions from the buffer and has been traced to cystine and cysteine in protein hydrolysates or —SH— and —S—S— groups in protein. (One molecule of cystine is reduced at the dropping-mercury electrode to two molecules of cysteine.)

The author describes how to obtain a master curve for catalytic waves of cysteine in a given buffer. This serves to calibrate determinations of cysteine in protein hydrolysates and cystine in urine. Cysteine forms a stable complex with iodoacetate ($RS.CH_2COO^-$) and in mixtures this eliminates the contribution of cysteine leaving that of cystine unchanged. The Brdička reaction has also been studied for glutathione and acids containing a sulphydryl group. Disulphides like thioctic acid are reduced first at the dropping-mercury electrode.

The work on the reaction applied to native and denatured proteins has been reviewed, and a number of practical (clinical) applications have been noted. An unusual effect has been developed into a versatile analytical procedure.

Professor Glick has over the years shown excellent judgement in selecting topics for review and in finding the right contributors. Biochemical analysis seems, from the portents of this volume, to be needed on a vast scale which requires more and more instrumentation, and indeed automation. The effort in terms of money, time and ingenuity which is now being put into highly sophisticated analytical work leaves one a little breathless. No doubt the information gained will be digested and properly interpreted. In the past a great many advances in Science have come from somewhat amateurish insights. We are now giving a fair test to the notion that aspects of the process of discovery can be mechanised, and it may be that by processing overwhelming masses of factual information great new ideas will emerge. Who can say? R. A. MORTON

ORGANIC CHEMISTRY. Volume I. THE FUNDAMENTAL PRINCIPLES. By I. L. Finar, B.Sc., Ph.D., A.R.I.C. Fourth Edition. Pp. xvi + 853. London: Longmans, Green & Co. Ltd. 1963. Price 50s.

Learning organic chemistry becomes, with the advance of the science, less and less the memorising of types of compounds and methods of preparation and more and more one of acquiring knowledge of the underlying principles of molecular structure and reaction. With such knowledge, the modern organic chemist engaged, say, in drug research, finds it almost easier to make his complicated compounds than to name them.

Finar's "Organic Chemistry" is, and has been for some time, a standard text-book for degree and honours students; four editions and ten impressions in 12 years preclude any doubt on this point. A review might therefore seem supererogatory, the merits of the work being so well recognised. However, a comparison of the various editions shows how the need for new editions arises from the intrusion of new ideas into the science. These intrusions become, in time, some of the fundamental principles, the description and application of which is the aim of the work. Such comparison also high-lights the amount of labour involved in keeping a text-book apace with new developments; it is far from being a matter of corrections.

The arrangement of the subject matter is conventional. After an introduction and a chapter on the structure of the atom (which deals to an equal extent with the molecule and its orbitals), aliphatic compounds are dealt with in fifteen chapters, alicyclic in one, aromatic compounds in ten and heterocyclic in two. One of the aliphatic chapters is devoted to compounds of sulphur, phosphorus, arsenic and silicon and one to organo-metallic compounds.

The distinctive features of the work are the dissertations, interspersed at appropriate places in the chapters, on the fundamental principles involved. Particular stress is laid on molecular structure, and reaction mechanisms are considered from the point of view of electronic theory as, for example, in the electrophilic, nucleophilic and free-radical mechanisms of aromatic substitution. New matter includes, among other subjects, molecular shape and reactivity, transition state theory of reactions, neighbouring group participation and molecular over-crowding. With the ever expanding range of new compounds being made, some organised system of nomenclature becomes essential and this is dealt with appropriately in each chapter and an appendix.

Although the book is primarily intended as a students text-book, up-to-dateness in fundamental theory makes it equally useful for the trained organic chemist, though it naturally has its limitations with respect to the types of compound described, *e.g.*, heterocyclic compounds are restricted to six-membered rings and condensed aromatic rings to three-ring systems. These, however, are matters not introducing new principles and more appropriate to text-books on advanced organic chemistry.

One of the best tributes to the work was its adoption for his students by the late Professor T. S. Wheeler, himself part author of a one-time best seller among text-books on the same subject.

J. I. M. JONES

GAS CHROMATOGRAPHY. Edited by NATHANIEL BRENNER, JOSEPH E. CALLEN and MARVIN D. WEISS. Pp. xxiv + 719. New York and London: Academic Press Inç. 1962. Price 157s.

This book comprises the proceedings of the 3rd International Symposium on Gas Chromatography held at Michigan State University under the auspices of the Instrument Society of America, from June 13th to 16th, 1961.

The first and main part of the book contains the 37 papers that were presented. These are followed by an Appendix recording the Discussion and finally a Bibliography on Gas Chromatography is given. This is a continuation of two previous bibliographies and covers the period June, 1960, to October, 1961.

A short introduction is written by Golay, in which he emphasises the important developmen that have taken place in column and detector design, and he stresses the need for still more sensitive detectors and suitable column improvements that will enable us to detect large organic substances.

The papers appear in a sequence such that those dealing with topics having something in common are grouped together. The Discussion follows a similar pattern and is arranged to deal with groups of papers at the same time. The first 4 papers deal mainly with a study of the effect of the nature of the liquid phase and the amount used on the solid support on separations. The nature of the solid support has been studied and the elimination of tailing effects by treatment of the support to render its surface inactive is clearly demonstrated. The advantages gained by using a lower column temperature, with liquid loading on the inert support drastically reduced, are demonstrated in a paper dealing with efficiency of columns. Programmed-temperature gas chromatography has not been neglected, and in one paper a theoretical study of the method has been made. There is also an interesting paper on a study of the effect of various factors on column permeability.

Halasz, who is well known for his work on the use of capillary columns for the quantitative analysis of hydrocarbon mixtures, presents a further paper on this subject. In an interestin paper on the performance of coated capillary columns, Desty, Goldup and Swanton express surpris that the application of these columns has not been more widely appreciated. In the opinio of the reviewer this lack of more general use in quantitative work may be due to the high cost o unavailability of the special integrating and recording apparatus. Two further papers deal wit the application of gas chromatography to relatively high-boiling petroleum and tar oils and pol phenyl ethers.

After a specialised paper on the measurement of absorption isotherms by high temperatu gas chromatography, there follows a series of 9 papers on the role of detectors in gas chromat graphy. Most attention seems to have been given to the now well known flame-ionisation detect with particular emphasis to its response to functional groups. The plot of carbon number again response (relative area per molecule) is shown to be linear for a wide range of classes of compoun Similar investigations for the argon detector are reported.

It is evident that the search for new types of detector still continues. In one paper a no method based on the use of ultrasonics is put forward, and it shows that an "ultrasonic whistl holds promise. The use of a low-current electric-discharge detector has been explored, and detector based on a thermionic diode operating in an inert gas at atmospheric pressure has bee examined.

The originator of the argon detector, Lovelock and co-author Gregory, have contribut a paper on their most recent work with the electron-capture ionisation detector.

Probably the most novel paper is one that deals with the development of a Lunar G Chromatograph designed to telemeter information on the composition of possible organics a volatiles present on the moon's surface. By means of a probe, a suitable sample would be remov from the moon, hydrolysed and the products submitted to gas chromatography.

Sample-introduction and fraction-collection systems are considered in other papers, and final there are 10 papers dealing with miscellaneous applications. These include the analysis of hyd carbon mixtures, fluoralcohols, fluoracrylates and flavours.

It is doubtful whether the Bibliography at the end of the book serves a useful purpose any worker who possesses the Gas Chromatography Abstracts issued by the Gas Chromatogra Discussion Group (Butterworth & Co., London).

The book is well produced and should certainly be included in the library of all those interest in gas chromatography.

A. F. WILLIAMS

HANDBOOK OF ANALYTICAL CHEMISTRY. Edited by LOUIS MEITES. Pp. xvii + 1806. Ne York, Toronto and London: McGraw-Hill Book Co. Inc. 1963. Price £18 8s.

Someone has said that science is measurement, which is, perhaps, a particularisation o Horace's "Est modus in rebus"—there is measure in everything. This needs no elaboration fo the analytical chemist who is faced not only with the daily task of measurement, but with the equally frequent need, if he is to complete his task daily, to refer to measurements made in th past by others of his profession. It is easy to understand this need in these days of electrometric nuclear-magnetic and thermo-analytical techniques, but not so readily appreciated that it wa already felt more than 80 years ago.

It was met some time before 1880 by the publication of the "Chemiker Kalendar" which by 1930 contained some 1500 pages of the kind of quantitative information required by the practising chemist. Then, about the time of the First World War, we had, in English, Atack's Chemists Handbook and the American Handbook of Chemistry and Physics with its current 4000 pages covering a wider and more general field. In more sectional fields were the Landolt - Bernstein physico-chemical tables and Seidell's solubility tables. Now, in the present volume the analytical chemist comes into his own in a compendium extending to over 1800 pages of the fundamental data and techniques useful in his work. The compilation is the work of some 140 expert chemists and is published by a firm who ought to know what they are about, having published seventy-five such handbooks from plumbing, piping and petroleum to physics and chemistry.

To review such a varied collection of data as is presented in this volume is impracticable; one can but mention some of the topics to illustrate the scope of the work. Thus, the section on qualitative analysis contains, besides the classical H_2S scheme of cation identification, eight alternative schemes, with another nine for anion identification and sixteen covering organic qualitative analysis. The fifteen sections into which the book is divided include sections on electrometric, optical, nuclear and magnetic, thermo-analytical and biological (mainly microbiological) techniques. Section 3, on inorganic gravimetric and volumetric analysis, includes acid - base titrations in non-aqueous media, precipitation, redox and complexometric titrations with exhaustive tables, for example, of 116 redox titration methods, fifty-four pages describing indicators used in complexometric titrations and a 26-page table of methods for determining inorganic cations by visual EDTA titrations.

One of the most interesting sections (179 pages) is that on techniques of separation, covering solvent extraction, distillation, the various forms of chromatography, electrophoresis and ion exchange. Another is that (294 pages) on methods for the analysis of technical materials. Working details are, of course, not given, but the principles, reagents and references are given for metals nd alloys, cement, fuels, fertilisers, oils and fats, paints, pesticides, petroleum and pharmaceutical products, soap and detergents, plastics, water and sewage.

Such a compendious volume needs a good index and this need is adequately supplied. Of the task of compilation, our French colleagues would say "C'est formidable," and the editor and his contributors are to be congratulated. The book is a valuable reference work for the analytical laboratory, particularly for the occasional special sample which is outside the field of its normal work. The amount of information contained is no doubt worth the price charged, but this price, t the same time, is likely to limit its sale to the industrial analytical laboratory with a wide range of practice.

J. I. M. JONES

STRUCTURAL CARBOHYDRATE CHEMISTRY. By E. G. V. PERCIVAL, D.Sc., Ph.D., F.R.I.C. Revised by ELIZABETH PERCIVAL, D.Sc., Ph.D., F.R.S.E., with a Foreword by E. L. HIRST, C.B.E., F.R.S. Second Edition. Pp. xvi + 360. London: J. Garnet Miller Ltd. 1962. Price 40s.

This important text-book of carbohydrate chemistry was first published by the late Dr. E. G. V. Percival some twelve years ago. Since that time there has been an enormous growth in the volume of knowledge in the carbohydrate field, and Dr. Percival's wife has now extensively revised this book.

In the first four chapters some minor but important changes have been made. For example, Hudson's isorotation rules and a new section on sugar conformation are included, together with some comments on the structure of phenylozazones, and a worthwhile and timely section on nitrogen-containing or amino deoxysugars. In other chapters, particularly on new disaccharides, a good deal of more recent information has been provided, and with this is included, for the first time in a text-book I believe, the recent syntheses of disaccharides.

A completely new chapter on oligosaccharides has been provided and an enormous amount of knowledge in this ever-expanding subject has been put into a concise form. Included in the oligosaccharide chapter is new knowledge of oligosaccharides that are derived from sucrose, the important human-milk oligosaccharides and the enzyme-produced Schardinger dextrins. Considerable new information is provided on the very important nitrogen glycosides and on the polyhydroxy compounds, the inositols, which are related to carbohydrates.

The whole of the section on the polysaccharides has been rewritten, and those of great biological importance have been included. It is difficult to get enough information in a short space on the complex polysaccharides to give to students the right kind of picture of these important compounds, but by concise writing and by picking out the most significant information which only the specialist can do, it has been possible to make the whole subject quite exciting. Much knowledge is provided on the enzymic synthesis and breakdown of polysaccharides and important sugars of animal origin, especially those containing nitrogen and sulphur, and known as the "mucopolysaccharides," have been dealt with in a concise and orderly fashion.

This is a text-book written for advanced students in chemistry and it does achieve its purpose in a remarkable way. It gives, in a relatively small space, a well presented and orderly fashioned account of all important groups of the saccharides. It is beautifully printed and well supplied with subject index, and the formulae are clear and well drawn. Altogether this book can be recommended for all libraries and for all advanced and research students in chemistry. The book continues the high tradition set by the late Professor Sir Norman Haworth and his School of Chemistry at Birmingham, of which the Percivals were prominent members. M. STACEY

INORGANIC ISOTOPIC SYNTHESES. Edited by ROLFE H. HERBER. Pp. x + 249. New York: W. A. Benjamin Inc. 1962. Price $7.50.

The availability, both of ample supplies of radioactive isotopes of most of the elements and of an increasing number of mass spectrometers, has led to a revival of interest in isotopic-dilution analysis and other tracer techniques. For these, a prerequisite is a supply of the compounds of interest, isotopically "labelled."

Although in many instances nowadays suitable labelled compounds are commercially available, some workers still prefer to prepare their own: when the compounds are not otherwise available they have, of course, no alternative. Some of the information required by the chemist who undertakes isotopic syntheses, is gathered in book form; for example, "Isotopic Carbon" by Calvin *et al.*, but the bulk of it is scattered in journals.

The book under review is intended as a guide to part of the field, that of the preparation of inorganic compounds labelled with deuterium, tritium, nitrogen-15, oxygen-18, phosphorus-32, sulphur-35, chlorine-36 and iodine-131. It is claimed that in many instances enough detail is provided to make consultation of the literature unnecessary, and in other instances, especially when "unstable or esoteric substances" are involved, reference is given to the appropriate original publications.

The first chapter, on General Principles, deals with such subjects as Radiochemical Manipulations, Radioassay and Mass Spectrometry. Statements here cast doubt on the Editor's claim to have inveigled the best qualified person in each field to contribute. For example, in discussing the radiations likely to be encountered by a user of radioisotopes, the implication (p. 3) that gamma radiation is less likely from fission products than from nuclear-reactor produced nuclides is, at the very least, misleading and the statement (p. 6) that in a Geiger - Müller counter complete breakdown of the gas occurs with each ionising event, is untrue.

Succeeding chapters, which are in effect separate monographs, deal in turn with synthesis of compounds of each of the isotopes listed above.

The book has been produced from typescript by photolithography, possibly to save time, as haste is evident in the many irritating errors and omissions. Although some of these, such as the writing of NaO_2 for sodium peroxide (p. 25) and AgF_2 for silver fluoride (p. 48) are admittedly trivial, the omission of dimensions from Fig. 1 (p. 123), when the text specifies operating conditions for a cell of "the dimensions given in Fig. 1," is more tiresome. Even more tiresome andun helpful, and certainly not in line with the declared aim of the Editor, is the provision of a reference to a "private communication" as the only information on the preparation of one compound (potassium carbonate-^{18}O by exchange, on p. 132).

No reference is made to the need for compounds of high, or at least known, purity for accurate results in, for example, isotope-dilution analysis, although other sources of error, such as the isotope effect, are mentioned. As it cannot be assumed that the compounds produced will always be of high purity, some information on purity checks might be expected; it is all but absent.

However, in spite of its faults, many of which could have been eliminated by a more careful revision, the book would be a valuable addition to the library of anyone engaged in isotopic syntheses. D. A. LAMBIE

NCYCLOPAEDIC DICTIONARY OF PHYSICS. Editor-in-Chief, J. THEWLIS. Volumes I to VI: Vol. I, pp. xvi + 800; Vol. II, pp. x + 880; Vol. III, pp. x + 894; Vol. IV, pp. x + 836; Vol. V, pp. x + 782; Vol. VI, pp. x + 883. Oxford, London, New York and Paris: Pergamon Press. 1962. Price per set of 9 volumes, £106; $298.

In 1922 the Dictionary of Applied Physics, edited by Sir Richard Glazebrook, was published five volumes, each of which was devoted to a defined branch of the subject. Forty years have ow passed, during which time many fundamental advances in physics, some having the most r-reaching practical consequences, have been made.

The publication of a new Encyclopaedic Dictionary of Physics, of which the first six volumes e available for review, is therefore opportune. Its appearance will be welcomed by all who have casion to use a technical library. The tendency towards unification of different branches of ience, in spite of their increasing complexity, has led to the inclusion of much subject matter [illegible] of, and outside, physics. Of particular interest to readers of *The Analyst*, apart [illegible] on the well-known physical tools of their calling, will be the contributions devoted matters in the fields of physical and structural chemistry, crystallography, physical metallurgy d radiation chemistry.

The order of entries throughout the sequence of volumes is strictly alphabetical, although e order of words in the title of each entry is sometimes chosen so as to place related topics in ntiguity. Thus consecutive entires in the fifth volume deal with pump, axial; pump, centrifugal; ump, circulating; pump, diffusion; pump, getter-ion and so on. This simple and useful device arrangement is to be commended. The actual entries range from three or four lines of definition half-a-dozen well illustrated pages for topics of major significance. The long monographs aracteristic of Glazebrook have inevitably been greatly reduced in length. Friction, for example, adequately treated in four pages compared with the sixty-three pages under this heading in the arlier dictionary. The value of the articles in the present work is, however, enhanced by suitable oss-reference to other entries and by useful bibliographies.

Essential features of a good encyclopaedic dictionary are authority, readability and "findility." A sampling technique, ranging between the limits at present provided—Abbé refractoeter to Stellar luminosity—indicates that the first two requirements are amply fulfilled. Judgment bout the third criterion must, for the present, be held in suspense. The publishers' foreword nd the editor's preface both stress the importance of consulting the Index Volume before searching the dictionary, because so many items "are not given separate entries, but are covered in other elevant articles." This advice is repeated in a footnote on every alternate page of each volume. n the absence of the index volume one cannot therefore report on "findability." Again using a ampling technique, only some 50 per cent. of a group of arbitrarily chosen topics were found y alphabetical search. The promised index is thus essential, and will form an integral part f the whole.

The publishers, Dr. Thewlis (editor-in-chief), his associate editors, the consulting editors and e many contributors are to be congratulated on the production of this outstanding work. The ourage and vision displayed in undertaking this onerous task have been fully justified by its [illegible]

B. S. COOPER

Publications Received

PHASE CHROMATOGRAPHY. VOLUME 1. GAS CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. SCOTT. Pp. viii + 199. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 42s.

PHASE CHROMATOGRAPHY. VOLUME 2. CAPILLARY CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. Scott. Pp. x + 120. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 35s.

GAS PHASE CHROMATOGRAPHY. VOLUME 3. TABLES FOR GAS CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. SCOTT. Pp. x + 162. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 40s.

INFRA-RED SPECTROSCOPY AND MOLECULAR STRUCTURE: AN OUTLINE OF THE PRINCIPLES. Edited by MANSEL DAVIES. Pp. xiv + 468. Amsterdam, London & New York: Elsevier Publishing Company. 1963. Price 75s.

Mises au Point de Chimie Analytique Organique, Pharmaceutique et Brom
Edited by J.-A. Gautier and P. Malangeau. Onzième Série. Pp. 252. P
et Cie. 196[illegible]. Price 55 NF.

L'Analyse Qualitative et les Réactions en Solution. By G. Charlot. Fi
Pp. xvi + 442. Paris: Masson et Cie. 1963. Price (paper) 65 NF; (cloth) 75 N

The Modern Structural Theory of Organic Chemistry. By Lloyd N. Ferg
viii + 600. Englewoods Cliffs, N. J.: Prentice-Hall, Inc.; London: Prentice
national, Inc. 1963. Price 104s.

Infrared Band Handbook. Edited by Herman A. Szymanski. Pp. xii + [illegible]84.
Plenum Press. 1963. Price $35.

Recent Progress in Microcalorimetry. By E. Calvet and H. Prat.
from the French by H. A. Skinner. Pp. xii + 1[illegible]7. Oxford, Lon
Paris: Pergamon Press. 1963. Price 60s.

Spektrale Zuordnungstafel der Infrarot-Absorptionsbanden. By Dr.
Pp. 18. Berlin, Göttingen and Heidelberg: Springer-Verlag. [illegible] Price

Boron Hydrides. By William N. Lipscomb. Pp. x + 275. New York
W. A. Benjamin, Inc. 1963. Price $15·40.

Metal Ions in Aqueous Solution. By John P. Hunt. Pp. xii + 124. New York
Amsterdam: W. A. Benjamin, Inc. 1963. Price $6·05.

Errata

March (1963) issue, pp. 162, 163 (twice), 164 and 165, references. *For* "British Standa
3406 : Part 2 : 1962," *read* "British Standard 3406 : Part 2 : in the press."

Ibid., p. 174, last line, and p. 175, 13th line. *For* "British Standard 3406 : Part 3 : 196
read "British Standard 3406 : Part 3 : 1963."

Ibid., p. 179, 7th line. *For* "British Standard 3406 : Part 4 : 1962, p. 196" *read* "Briti
Standard 3406 : Part 4 : in the press."

October (1963) issue, p. 768, penultimate line. *For* "specific gravity", *read* "speci
activity."

November (1963) issue, p. 829, author's address. *For* "64–68" *read* "64–78."

Ibid., p. 833, reference 10. *For* "26" (the page number of the reference) *read* "36."

Ibid., p. 834, reference 23. *Delete* "Berry, M. M., and Kent, A."

Ibid., p. 899, reference 2. *For* "71" (the page number of the reference) *read* "76."

The Editor regrets the errors in D. R. Curry's Review Paper on Information Retrieval,
resulted from a misinterpretation of some corrected proofs.

Reprints from *The Analyst*

Analytical Methods Committee Report: "Determination
Chlortetracycline and Oxytetracycline"

The Report prepared by the Antibiotics Panel of the Additives in Animal
Sub-Committee, "The Determination of Penicillin, Chlortetracycline and Oxytetrac
Diet Supplements and Compound Feeding Stuffs," reprinted from *The Analyst*,
835–850, is now available from the Secretary, The Society for Analytical Chemistry
grave Square, London, S.W.1, price to members 1s. 6d. each; to non-members, 2s.

Review Paper "Thermogravimetric Analysis"

Reprints of the Review Paper, "Thermogravimetric Analysis," by A. W. Coats a
Redfern, published in this issue of *The Analyst*, will soon be available from the S
The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. p
post free.

A remittance for the correct amount, made out to The Society for Analytical Ch
st accompany every order; these reprints are not available through Trade Agen